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Studies in Cancer

VIII. Stilbestrol and Certain Steroids in Relation to Tumor Growth Resistance*

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INTRODUCTION

This report concerns resistance against tumor growth, as influenced by estrogens. In previous studies (11, 13) of the growth of implanted sarcoma 180 in pedigreed mice of (a) the black C57 strain and of (b) a Bagg albino A strain, both of which rarely showed spontaneous tumors, it was found that large doses of estrone could enhance previously induced "immunity."[1] This effect showed itself both in a lower percentage of successful inoculations and in a smaller mean size of successfully implanted tumors. The estrogen itself produced no significant effect upon either of these criteria.

In order to learn further whether the result was due to estrogenic activity or to a pharmacological nonspecific toxicity, similar experiments have been performed with related chemical substances; namely, with the nonsteroid diethylstilbestrol dipropionate[2] and with progesterone.[3] The experiments have been carried out with the low-tumor Bagg albino A strain, in the high-tumor Bar Harbor A strain, and in a low-tumor substrain of the latter. A few observations were conducted also in C57 black females.

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[1] It will be recalled that, as shown by W. U. Gardner and others, in murine strains in which estrone regularly produces cancer *large* doses of the hormone will suppress even the preliminary hyperplasia of the tissue.

[2] For the diethyistilbestrol dipropionate the authors are indebted to the Winthrop Chemical Company.

[3] For the progesterone and estrone the authors are indebted, respectively, to Roche-Organon, Inc. and to Parke, Davis and Company.

EXPERIMENTAL PLAN

The experimental method used was that described in a previous paper (11). The experimental animals were ordinarily divided into four subgroups, which were designated as follows:

1. Virulence controls
2. Immunity controls
3. Hormone alone
4. Hormone plus immunity

The method of handling these animals and the general conduct of the experiment were precisely that previously outlined (11).

In brief, the procedure was as follows. Sarcoma 180 was implanted *in the tails* of the "immunity control" animals and of the "hormone plus immunity" animals. After ten days the terminal portion of each tail, containing the small tumor, was amputated. Thereupon sarcoma 180 was implanted in the groins of these 2 groups and of the other 2 (control) groups simultaneously. Indeed, 1 animal from each of the 4 groups was inoculated seriatim, so that the control might be as perfect as possible.

In this fashion there resulted 4 groups of mice, all bearing implanted sarcomas in the groin. The "virulence control" and the "immunity control" mice received no further treatment. On the other hand, the "hormone alone" and the "hormone plus immunity" animals were injected with estrogen in large dosage 3 times a week. Thus 3 major variables were controlled, *i.e.*, the natural growth rate of the tumor implant, the inhibiting effect of the preliminary caudal inoculation, and the effect of the hormone itself.

The tumors were measured in 2 diameters twice a week. At each measurement, the incidence of total

"immunity" as evidenced by failure of tumor growth was noted. The animals were weighed at monthly intervals to exclude a possible nutritional effect on tumor growth.

Dosage of Hormone Used

In the case of the stilbestrol, the dosage used varied from 50 to 200 international units, per 22 gram mouse, administered subcutaneously approximately 3 times a week. The injections were begun at the time of the inoculation of the test tumor in the groin and were continued for at least 4 weeks. In some experiments injections were continued for 7 weeks. In the case of progesterone the dosage was 1 mgm. in sesame oil 3 times a week. In the case of estrone the dose was 100 to 200 international units in peanut oil twice weekly. In all instances the control animals were injected with an equivalent amount of the oily vehicle corresponding to the hormone preparation under study.

EXPERIMENTAL DATA

The experimental results represent studies on over 570 animals. Each tumor was ordinarily measured in 3 diameters twice a week. To conserve space, only mean diameters are presented in this report, and usually only mean diameters at maximal size (*i.e.*, after 3 to 4 weeks' growth). The data are arranged in relation to diverse strains of animal, and in relation to sex, as well as with respect to the effect of the particular hormone involved.

I. Studies in Strains Showing Infrequent Spontaneous Tumors

The observations on low-tumor strains were divided according to sex. This subdivision was made because it was not clear that stilbestrol would show estrogenic action in the male.

Females treated with stilbestrol and with progesterone.—In Table I are summarized data of experiments performed with the same strain of Bagg albino A mice used in the estrone experiments previously reported (11). In both Experiment 4 and Experiment 6, immunity was distinctly enhanced in the hormone group. In Experiment 6 the tumors in this hormone-treated group were also unusually small. As regards survival data, in Experiment 6, at the end of the 44th day, none of the original mice were alive and free from tumor in either the "virulence control" or the "hormone control" groups; and there were only 7 per cent of the immunity controls as against 23 per cent of the hormone-treated immunized animals.

Table I: Effect of Diethylstilbestrol Dipropionate

Summary of Experiments 4 and 6. Bagg albino A female mice, low-tumor strain, treated with diethylstilbestrol dipropionate, 200 I.U. 3 times weekly.

	Virulence control	Hormone control	Immunity control	Hormone plus immunity
Original number of mice	29	29	42	44
No. of mice 28th day	19	18	26	36
No. of tumors	19	18	19	18
Average tumor size *	15.7 ± 5.2	16.1 ± 4.6	11.0 ± 3.5	7.4 ± 3.5
Percentage of takes	100	100	73	50
Percentage immune	0	0	27	50
No. dead	10	11	16	8
Mortality (per cent)	34	38	38	18
Percentage of survivors with tumors	100	100	73	50
Percentage of original mice alive, free of tumors	0	0	17	41
SURVIVAL DATA AT 44TH DAY, EXPERIMENT 6				
Original no. of mice	14	14	14	13
No. mice 44th day	2	2	8	6
No. of tumors	2	2	7	3
Mortality (per cent)	86	86	43	54
Percentage of survivors with tumors	100	100	87	50
Percentage of original mice alive, free of tumors	0	0	7	23

* Hormone-immunity vs. Immunity control (statistical evaluation): $P = 0.08$.

When the 144 animals in Experiments 4 and 6 are studied from the standpoint of their status at the end of the 28th day, several interesting differences appear: (a) None of the animals were free from tumor unless immunized, but the hormone apparently increased the percentage of immunity from 27 to 50 per cent. (b) At this time the mortality was between 34 to 38 per cent for all groups except the hormone-immunity group, in which it was only 18 per cent. (c) Of the survivors, all had tumors in the "virulence controls" and "hormone controls," and whereas the "immunity

controls" showed 73 per cent tumors, those receiving hormone in addition to immunity showed only 50 per cent with tumors. (d) As regards the percentage of the original animals alive and free of tumor, there were none in the "virulence" and "hormone control" groups, and whereas there were only 17 per cent in the "immunity controls," there were 41 per cent in those treated with immunity plus hormone.

Progesterone.—In Experiment 2 the effect of progesterone in Bagg albino A females was found to be essentially negative. The "immunity control" animals were given 0.1 cc. of sesame oil subcutaneously 3 times weekly. The mice in 2 other groups, *i.e.,* "hormone" and "hormone plus immunity" were give 0.1 cc. of proluton (Schering) containing 1 mgm. of progesterone 3 times weekly. As shown in Table I A, the effect

TABLE I A

EFFECT OF PROGESTERONE

Experiment 2. Bagg albino A female mice, low-tumor strain, treated with progesterone, 1 mgm. 3 times weekly.

25th day	Virulence control	Progesterone control	Immunity control	Progesterone plus immunity
No. of mice	15	14	12	14
No. of tumors	15	14	7	5
Mean diameter	13.9	13.2	12	10.4
Percentage of takes	100	100	58	43
Percentage immune	0	0	42	57
Average weight (gm.)	20.6	20.0	23.8	18.8

SURVIVAL AFTER 8 TO 10 WEEKS

56th to 70th day	Virulence control	Progesterone control	Immunity control	Progesterone plus immunity
Initial No. of mice	15	15	15	15
No. of mice	3	0	7	8
No. of tumors	2	0	2	1
Survival (per cent)	9	0	47	53

was found to be essentially negative. On the 76th day, to be sure, there were more of the treated animals alive than of the immunized controls but the difference was probably not significant.

Males treated with stilbestrol.—In the experiments with diethylstilbestrol dipropionate the results of Experiments 3 and 5, dealing with males of the Bagg albino A strain, disclosed no great benefit from stilbestrol therapy (Table II). This result suggests a difference between the sexes. Such a difference might be expected in view of the general lack of pathological evidence of a specific effect of this drug in the male (7), as compared with smaller dosages in the female.

The data of the combined experiments indicate approximately the same degree of immunity whether or not the hormone was added. The gross mortality and the percentage of survivors free from tumor were slightly more favorable when hormone was used. There was some indication, also, that the effect of hormone alone in Experiment 5 might have been slightly beneficial as regards size of tumor. In general, however, these effects were of minor significance at best.

The obvious interpretation is that the stilbestrol failed to excite an estrogen effect in these murine males. To test this point a pathological study was made in Experiment 5 of histological sections of various organs, including adrenals and testicles. The material so examined failed to show objective evidence of the massive doses of hormone employed. This conclusion is in accord with the lack of effect on tumor growth and with other pathological studies in the literature (10). Indeed, the mean testicular weight for animals treated with stilbestrol for immunity controls was 88 mgm. as against 89 mgm. (Table II A).

II. Studies in Strains Showing Frequent Spontaneous Tumors

Experiments comparable to the preceding were performed upon hosts from strains showing a high incidence of spontaneous tumors. Both sexes were used; separately in some experiments, mixed in others. The animals used were about 3 months old at the start of the experiment.

Studies of stilbestrol in Bar Harbor albino A, high-tumor substrain.—In Table III are presented the summarized results of Experiments 7 and 8 dealing with the effects of diethylstilbestrol dipropionate in the females of this high-tumor strain. The table shows the dosage used, the relative number of takes, and the mean tumor diameter at 3 to 4 weeks for each of the 4 categories previously outlined, which control both immunity and hormone factors. In general, the data indicate that the principal inhibitory effect was that of the immunity. The hormone alone had relatively little effect, if any, and the hormone likewise failed to show a synergistic effect with the immunity.

Males.—In Table III A are shown similar data for males of the high-tumor substrain. Here again the result of hormone administration was not significant.

Survival of host animals.—Further information was accumulated in the form of data showing the survival of animals in various categories. In particular, attention was paid to the gross mortality, to the percentage of the survivors bearing tumors, and to the percentage of original animals alive and free from tumor. This last figure represents the net salvage. The time after the test inoculation at which such comparison was made was at about 7 weeks, although in some cases intercurrent epidemics prevented observations being continued that long.

In general these results indicate that the immunization alone explained the longevity of the treated mice (Tables III and III A). Indeed, repeated doses of the hormone tended to shorten the expectation of life.

Estrone in high-tumor substrain.—Experiment 9, shown in Table IV, presents an interesting contrast to the results previously obtained with estrone (11) in strains having a low incidence of spontaneous tumors. For this experiment 62 male and 73 female mice of the Bagg A albino strain were used. The summarized results are recorded in Table IV. At the end of the fourth week, 25 and 29 per cent of the "immunity control" and "hormone plus immunity" male groups, respectively, were immune. The tumor size in the two groups was identical. The ultimate salvage, measured at the end of the tenth week, indicated that the theelin did not enhance induced immunity; the actual values were 44 and 36 per cent for the "immunity control" and "hormone plus immunity" groups respectively.

Similar results were obtained in the female mice. The percentages of immune animals were identical,

TABLE II: EFFECT OF DIETHYLSTILBESTROL DIPROPIONATE

Summary of Experiments 7 and 8. Bagg albino A male mice, low-tumor strain, treated with diethylstilbestrol dipropionate every 3 days, 100 I.U. for 9 doses; thereafter 200 I.U. for 4 doses.

	Virulence control	Hormone control	Immunity control	Hormone plus immunity
Exp. 3, Bar Harbor albino A, low-tumor strain, 25th day				
No. of mice	14	12	15	11
No. of tumors	14	12	12	10
Percentage of takes	100	100	80	91
Percentage immune	0	0	20	9
Average tumor size (mm.)	19.3	17.8	11.1	7.6
Immunity control vs. hormone control (statistical evaluation): $P = 0.05$				
Virulence control vs. hormone control (statistical evaluation): $P = 0.45$				
Exp. 5, Bar Harbor albino A, low-tumor strain, 28th day				
No. of mice	13	18	22	23
No. of takes	13	18	5	5
Percentage of takes	100	100	23	22
Percentage immune	0	0	77	78
Average tumor size (mm.)	13.3 ± 4.3	9.4 ± 2.7	..	..
Mean mouse weight (grams)	25.8	25.6	26	26
Virulence control vs. hormone control (statistical evaluation) $n = 29$; $s = 3.6$; $t = 2.7$; $P = 0.01$				
Combined Experiments 3 and 5				
No. of mice	27	30	37	34
No. of takes	27	30	17	14
Percentage of takes	100	100	46	41
Percentage immune	0	0	54	59
Survival data on combined Exps. 3 and 5, 40th day				
Original no. of mice	37	34	41	40
No. of mice	11	17	30	32
No. of tumors	11	16	11	10
Mortality (per cent)	70	53	27	20
Percentage of survivors with tumors	100	94	37	31
Percentage of original alive, free from tumor	0	3.0	46	55

TABLE II A: COMPARATIVE EFFECT OF ESTRONE AND DIETHYLSTILBESTROL DIPROPIONATE ON TESTICULAR WEIGHT

Hormone used	Strain of mice		Virulence control	Hormone control	Immunity control	Hormone plus immunity	
						50 I.U.	
Stilbestrol	Bagg A albino	No. of mice	7	9	19	22	
		Mean testicular weight (mgm.)	54	75	89	88	
						50 I.U.	200 I.U.
Theelin	Black C57	No. of mice	12		24	24	24
		Mean testicular weight (mgm.)	87			61	31

TABLE III: EFFECT OF DIETHYLSTILBESTROL DIPROPIONATE

Summary of Experiments 7 and 8. Bagg Albino A female mice, high-tumor strain, treated with diethylstilbestrol dipropionate every 3 days, 100 I.U. for 9 doses; thereafter 200 I.U. for 4 doses.

	Virulence control	Hormone control	Immunity control	Hormone plus immunity
Exp. 7, Bar Harbor albino A, high-tumor strain, 28th day				
No. of mice			13	13
No. of tumors			4	3
Percentage of takes			31	23
Average tumor size (mm.)			9.3	7.4
Exp. 8, Bar Harbor albino A, high-tumor strain, 29th day				
No. of mice	20	21	15	19
No. of tumors	20	21	7	14
Percentage of takes	100	100	46	73
Average tumor size (mm.)	18.1	17.8	14.5	13.2
Combined Exps. 7 and 8, 28th day				
No. of mice	20	21	28	32
No. of tumors	20	21	11	17
Percentage of takes	100	100	39	53
Percentage immune	0	0	61	47
Average tumor size (mm)				
Combined Survival data on Exp. 7, 40th day, and Exp. 8, 52nd day				
Original number of mice	23	23	30	33
No. of mice	5	3	22	18
No. of tumors	5	3	6	6
Mortality (per cent)	79	87	27	45
Percentage of original mice surviving with tumors	100	100	27	33
Percentage of original mice alive, free of tumors	0	0	53	36

TABLE III A

Summary of Experiments 7 and 8. Male mice, high-tumor strain, treated with diethylstilbestrol dipropionate, 200 I.U. 3 times weekly.

	Virulence control	Hormone control	Immunity control	Hormone plus immunity
Exp. 7, Bar Harbor albino A, high-tumor strain, 28th day				
No. of mice			13	10
No. of tumors			8	4
Percentage of takes			62	40
Average tumor size (mm.)			5.9	11.4
Exp. 8, Bar Harbor albino A, high-tumor strain, 29th day				
No. of mice	22	22	20	20
No. of tumors	22	22	13	11
Percentage of takes	100	100	65	55
Average tumor size (mm.)	17.7	16.2	12.1	11.9
Combined Exps. 7 and 8, 28th day				
No. of mice	22	22	33	30
No. of tumors	22	22	21	15
Percentage of takes	100	100	64	50
Percentage immune	0	0	36	50
Average tumor size (mm.)	17.7	16.2	9.8	11.8
Survival data on Exp. 7, 40th day				
Survival data on Exp. 8, 52nd day				
Original number of mice	22	22	33	30
No. of mice	6	2	27	20
No. of tumors	6	2	13	5
Mortality (per cent)	73	91	67	76
Percentage of survivors that have tumors	100	100	63	25
Percentage of original alive, free of tumors	0	0	42	50

i.e., 35 per cent, in both the "hormone plus immunity" and "immunity control" groups at the end of the fourth week. The ultimate salvage, as measured at the end of the tenth week, again was lower in the group receiving estrone in addition to immunization. This failure of estrone to enhance induced immunity is in agreement with results obtained when diethylstilbestrol was used, as noted above. The data suggest that in this strain of mice, showing a high incidence of spontaneous tumors, female sex hormones are unable to increase induced immunity.

Progesterone in high-tumor substrain.—For this experiment 91 male mice of the Bagg A albino strain and 96 female mice of the same strain were used. Table IVA summarizes the experimental data. In the females at the end of the fourth week the "immunity control" group manifested a higher percentage of immunity and a slightly smaller mean tumor diameter than the "hormone plus immunity" group. At the end of the eighth week, however, 50 per cent of the "hormone plus immunity" group was alive and free from tumor, as against 32 per cent of the "immunity control" group. In the male animals at the end of the fourth week the percentage of animals immune and the mean tumor diameter were appreciably more favorable in the "immunity control" than in the "hormone plus immunity" group. As far as the ultimate salvage, measured at the end of the eighth week, was concerned, the "immunity control" group again showed a higher percentage than did the "hormone plus immunity" group. Thus, in brief, progesterone had no beneficial effect in the male animals but in the females it appeared to produce an incidental increase in the percentage ultimately salvaged.

TABLE IV: EXPERIMENT 9: HIGH-TUMOR MICE (BAGG A ALBINO) TREATED WITH ESTRONE

	Virulence control	Hormone control	Immunity control	Hormone plus immunity
		MALES		
Original number of mice	16	16	16	14
At end of 4th week:				
No. of mice	16	16	12	12
No. with tumors	16	16	12	9
Percentage with tumors	100	100	75	64
Percentage immune	0	0	25	36
Average tumor size (mm.)	14.4	14.3	8.0	7.9
At end of 10th week:				
No. of mice	2	0	10	8
Mortality (per cent)	87	100	38	43
Salvage (per cent)	0	0	44	36
		FEMALES		
Original number of mice	19	20	17	17
At end of 4th week:				
No. of mice	19	20	17	17
No. with tumors	19	20	11	12
Percentage with tumors	100	100	65	70
Percentage immune	0	0	35	30
Average tumor size	12.5			
At end of 10th week:				
No. of mice	5	1	11	8
Mortality (per cent)	74	95	35	53
Salvage (per cent)	0	0	47	29

DISCUSSION

The tentative indications of these studies are twofold. First, it is possible that animals with a high incidence of spontaneous tumors cannot have their immunity mechanism enhanced by an estrogen. Because several investigators have suggested that cancer is the result of a perverted sterol metabolism (2), such a finding would be highly significant. Secondly, as regards the effect of the estrogen, stilbestrol, the indication is that if it is effective in enhancing immunity against neoplasms this result occurs only by virtue of its transformation through female enzyme systems. In other words, it acts only because it is the precursor of an estrogen. This hypothesis requires more general information as to the metabolism of stilbestrol and its mode of action in the female.

TABLE IV A: EXPERIMENT 10: HIGH-TUMOR MICE (BAGG A ALBINO) TREATED WITH PROGESTERONE

	Virulence control	Hormone control	Immunity control	Hormone plus immunity
		MALES		
Original number of mice	23	24	22	22
At end of 4th week:				
No. of mice	22	22	21	22
No. with tumors	21	22	6	10
Percentage with tumors	91	96	27	45
Percentage immune	9	4	73	55
Average tumor size (mm.)	16.9	20.6	9.1	11.3
At end of 8th week:				
No. of mice	7	0	18	17
Percentage of mortality	70	100	18	23
Percentage of salvage	4	0	68	55
		FEMALES		
Original number of mice	26	24	22	24
At end of 4th week:				
No. of mice	26	23	21	23
No. with tumors	26	21	9	14
Per cent with tumors	100	88	41	58
Per cent immune	0	12	59	42
Average tumor size (mm.)	19.4	20.3	8.8	9.7
At end of 8th week:				
No. of mice	0	0	10	17
Percentage of mortality	100	100	55	29
Percentage of salvage	0	0	32	50

A survey of the recent literature indicates that hormones may influence the growth of several implanted mammalian tumors. A few instances may be cited. Eisen (4) found that subcutaneously administered estrogen inhibited an adenocarcinoma of the breast in rats. In mice, Zondek, Zondek, and Hartoch (16) checked the growth of a mammary adenocarcinoma with pituitary gonadotropic hormone. Wright, Klinck, and Wolfe (15) noted in rats a similar inhibiting effect of estrogens on spontaneous fibroadenoma. Likewise, Andervont (1) reported that female mice that survived implantation were more resistant than males to subsequent implantation. Gross (8) also noted a sex difference in resistance to transplantable tumor.

In general, the doses of hormone used in the various experiments cited were huge. Thus Eisen (4) gave 1,000 R. U. of estradiol twice weekly, using tumor R2426. The doses employed in the present study likewise were large. Therefore it is conceivable that the effects produced were indirect, *e.g.,* through the pituitary. That they were not due to poor general nutrition was indicated by controlled studies of the rate of growth.

The difference between strains and substrains described in the present report partially explains why other investigators have attained negative effects. For example, Emge, Murphy, and Schilling (6) found that estrone had no effect upon benign transplantable mammary tumors in the rat. Likewise Bischoff and Maxwell (3) observed no effect of estrogenic hormone on sarcoma 180. Indeed, Orbison and his associates (12) even separated by selective breeding two strains of rats such that one was susceptible and the other resistant to implants of induced sarcoma. Orbison decided that the response was determined largely by heredity. Somewhat the same point of view was advanced by Eisen and Woglom (5), who concluded that acquired resistance is not directed specifically against the malignant cell, but depends upon genetic differences between the animal inoculated and the one originally producing the neoplasm. The present study indicates that even in the same strain foster nursing can produce a substrain that will react differently with respect to hormonal inhibition of tumor growth. This is true even when the parent tumor used for inoculation arises spontaneously in a closely related animal of a highly inbred stock.

Analogous results were found by Law (9), working with transplantable leukemia. He found that the resistance of refractory mice toward these malignant cells was reduced by the foster nursing of the refractory mice by mothers of the susceptible strain during the first 6 to 7 days of lactation. The converse (when the strains were reversed), however, was not true. In a subsequent communication (14), the present authors will present evidence that a similar effect can be produced by changing the source of the inoculated or implanted tumor. These various experiments indicate that several different factors influence resistance against the growth of malignant tissue. Among these are heredity, endocrine status, the "milk factor," and the origin of the tumor.

As regards the sex difference noted in this report for diethylstilbestrol, the results harmonize with those of Lipschütz, Vargas, and Palma (10), who compared natural and artificial estrogens with respect to the fibroid tumorous reaction of the guinea pig. These authors, using both the stilbestrol and hexestrol type of synthetic estrogen, found a decided effect in females but a minimal effect in males.

CONCLUSIONS

Comparative studies of the effectiveness in inhibiting the growth of implanted sarcoma 180 of three estrogens, *i.e.,* estrone, diethylstilbestrol, and progesterone have been made. In certain strains, estrone is effective both in males and females; in such strains stilbestrol is effective in females but not in males. Progesterone, however, is not effective in rather high dosage.

The favorable effects observed appear to consist in enhancement of induced tissue "immunity" or resistance to tumor growth.

In certain substrains having a high incidence of spontaneous tumor these estrogens are ineffective, even though the corresponding low-tumor substrains showed beneficial results from the administration of estrogen. This finding suggests that the "milk factor" is involved in resistance to tumor growth.

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Studies in Cancer

X. Oxidative Capacity of Tumors*

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In previous papers (2, 10) from this laboratory have been reported studies of a series of human and animal neoplasms, concerning biochemical activity in relation to their pathological status. These chemical studies included the cytochrome-oxidase system and succinoxidase activities in tumor slices. Observations were made with the following tumors: sarcoma 180, Yale tumor No. 1, rhabdomyosarcoma, lymphosarcoma C_3HED, *p*-dimethylaminoazobenzene hepatoma, and a series of human neoplasms.

The Q_{O_2}, that is, the oxygen uptake, of all these tumors was of the same order of magnitude as that of the homologous normal tissues. Their behavior towards paraphenylenediamine and succinate, however, was very different. Whereas the oxygen uptake of the normal tissue slices studied increased definitely, *i. e.*, up to +250 per cent in the presence of the substrates mentioned above, the tumor slices failed to respond or responded very little. This low responsiveness seems to be a characteristic feature of neoplasia. Obviously, the phenomenon may be caused by (a) deficiencies in enzyme systems, or (b) by differences in the permeability to the given substrates (paraphenylenediamine or succinate), or (c) by the loss of a coenzyme factor. To elucidate this point, the behavior of tumor homogenates was compared with the homogenates of homologous tissues. The tissues studied were *p*-dimethylaminoazobenzene hepatoma, rhabdomyosarcoma, lymphosarcoma, Yale tumor No. 1, V_2 carcinoma, normal rat liver, and normal rat and mouse leg muscle. Data concerning the behavior of V_2 carcinoma slices towards succinate and paraphenylenediamine are also recorded in this paper.

EXPERIMENTAL METHOD

The biochemical procedure followed in the present investigation was the measure of the oxidative capacity of tissue homogenates in the presence of paraphenylenediamine and succinate. The rate of oxygen uptake was measured at 37° C. The single vessel manometric method of Warburg (16) was used. The test systems used in the experiments to be described were homogenates of tumor or homologous tissue. The homogenates were prepared by grinding the tissues in ice-cold water in a homogenizer. The homogenized suspension was brought to pH 7.3 with phosphate buffer; the final concentration was 0.06*M*. The amount of tissue in the solution was generally 10 per cent for the tumors, 5 to 10 per cent in the case of muscle, and 2.5 to 5 per cent in the case of normal liver. When these concentrations were doubled, the rate of oxygen absorption in the first 30 minutes also was doubled. This fact indicates that the tissue suspension was not too dilute and that the concentration of the substrate was at its optimum.

The final concentration of succinate was 0.018*M*, and that of paraphenylenediamine was 0.009*M*, in the case of hepatoma and of the normal liver homogenates. In the case of the homogenates from other tumors and from muscle, experiments were performed both at the above concentrations and at twice these concentrations. In this latter case there was no increase in the rate of oxygen uptake; indeed, a rather small inhibitory effect was observed.

The materials used were the following: hepatoma produced in Wistar strain rats by means of *p*-dimethylaminoazobenzene; Yale tumor No. 1; rhabdomyosarcoma produced by methylcholanthrene; and lymphosarcoma C_3HED. The mouse tumors were propagated in pure strain mice, and V_2 carcinoma in brown domestic rabbits. As control tissues, normal rat liver and normal rat and mouse leg muscle were used. In sampling the tumor care was taken to remove all grossly necrotic tissue present.

Hepatoma Induced by *p*-Dimethylaminoazobenzene

Rats of the Wistar strain were fed exclusively the diet described by Kensler, Sugiura, and Rhoads (8). This diet contains, per kilogram, 20 cc. of olive oil with 3 per cent of *p*-dimethylaminoazobenzene, mixed with finely ground brown rice. A supplement of fresh carrots was supplied each day.

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** Finney-Howell Research Fellow.

It was shown in a previous paper (10) that during the first 50 days the succinoxidase and the cytochrome *c* activities of the livers of rats maintained on this diet remained high and may have even increased somewhat. About the 70th day, however, a rather rapid decline in the activity of each physiological unit set in, and eventually reached the low level characteristic of neoplastic tissues. The first definite tumor appeared on the 163rd day. At that time the succinoxidase and cytochrome system activities had reached the base line. In the present work the evolution of the malignant process was followed by determinations of the succinoxidase and cytochrome *c* system activities of liver slices. The findings were similar to those previously reported (10).

At the 180th day, when definite tumors had developed, the animals were sacrificed and the livers removed rapidly. The liver homogenate was prepared as described above. The final concentration of paraphenylenediamine was 0.009*M*, that of succinate was 0.018*M*. Control experiments were run with homogenized normal rat liver.

TABLE I: RESPONSE BY TISSUE HOMOGENATE TO SUCCINATE AND PARAPHENYLENEDIAMINE. CU. MM. OXYGEN UPTAKE PER MGM. DRY WEIGHT PER HOUR

No addition	Succinate 0.018*M*	Δ	p-$C_6H_4(NH_2)_2$ 0.009*M*	Δ
		p-DIMETHYLAMINOAZOBENZENE HEPATOMA		
1.8	2.6	0.8	1.9	0.1
1.8	5.2	3.4	1.4	—0.4
5.4	6.9	1.5	5.4	0.0
1.4 *	17.2	15.8	10.3	8.9
		NORMAL RAT LIVER		
1.5	30.8	29.3	17.3	15.8
1.0	24.6	23.6	11.6	10.6
2.4	24.0	21.6	12.0	9.6
3.6	30.1	26.5	14.8	11.2

* Cirrhosis of the liver.

The results are summarized in Table I. They show that homogenates of hepatoma respond very little to the substrates added whereas homogenates of liver present a very definite response. The results in column Δ express the increase in cu. mm. oxygen uptake for 1 mgm. dry tissue in 1 hour. They express merely the activity of the enzymic systems acting upon the added substrates under the given circumstances. In the case marked by an asterisk, in which a high response was noted, the histological diagnosis was cirrhosis of the liver, not cancer. This particular rat was resistant to the carcinogenic agent; the irritation led only to cirrhosis.

TRANSPLANTED MOUSE TUMORS

Observations were made with the following transplantable mouse tumors: Yale tumor No. 1, rhabdomyosarcoma produced by methylcholanthrene, and lymphosarcoma C_3HED. Yale tumor No. 1 was borne by Bar Harbor strain A, and by Strong strain A mice. The lymphosarcoma and the rhabdomyosarcoma were borne by Bar Harbor C3H mice.

The results with rhabdomyosarcoma and lymphosarcoma were contrasted with data from normal leg muscle of the same murine host and with that of the rat. The final concentrations of paraphenylenediamine were 0.009*M* and 0.018*M*, respectively. Those of succinate were 0.018*M* and 0.045*M*. The data (Table II) show that the tumor homogenates exhibited very little response to either substrate. Normal muscle homogenates, under the same conditions, showed a decided response.

V_2 CARCINOMA

This carcinoma, derived from virus-induced papillomas in domestic rabbits, has been described in detail by Rous and Beard (11) and by Rous, Beard, and Kidd (12). In brief, it appeared in the midst of papillomatous growths and is representative of only one type, *i. e.*, the squamous cell carcinoma. The author is much indebted to Doctor Rous for the original tumor.

In Table III are summarized the data concerning the oxidative capacity of V_2 carcinoma slices in mammalian Ringer solution containing 0.015*M* phosphate buffer, at pH 7.3 in the presence of paraphenylenediamine and succinate. The concentration of succinate was 0.018*M;* that of paraphenylenediamine was 0.009*M*. As indicated in the table, the response of V_2 carcinoma slices to succinate was only +31.5 per cent and to paraphenylenediamine about −8.9 per cent. This low responsiveness, therefore, harmonizes with the findings for other neoplastic lesions (2, 10). The table demonstrates, likewise, that the homogenized tumor shows a low oxidative capacity to both paraphenylenediamine and succinate.

COMMENT

Several investigators have reported that a deficiency of cytochrome *c* is characteristic of neoplasia (3, 5, 6, 7, 15). Other investigators have reported that several enzymes are poorly represented in tumor tissue. That certain new growths are low in succinic dehydrogenase was first reported by Elliott and Greig (4), and later by Potter (3, 13). Cytochrome oxidase also was reported to be low in tumors by Schneider and Potter (13) and by Shack (14). However, tumor slices possess the same oxygen uptake rate in Ringer solution as do the homologous tissue slices. If succinate or paraphenylenediamine are added, the rate of oxygen uptake by normal tissues increases up to +250 per cent, while the tumor slices fail to respond or respond very little.

TABLE II: RESPONSE BY TISSUE HOMOGENATE TO SUCCINATE AND PARAPHENYLENEDIAMINE. CU. MM. OXYGEN UPTAKE PER MGM. DRY WEIGHT PER HOUR

No addition	Succinate 0.018*M*	Δ	Succinate 0.045*M*	Δ	p-$C_6H_4(NH_2)_2$ 0.009*M*	Δ	p-$C_6H_4(NH_2)_2$ 0.018*M*	Δ
				YALE TUMOR				
2.0	3.6	1.6	3.3	1.3	3.0	1.0	2.0	0.0
1.6	3.8	2.2	3.0	1.4	2.7	1.1	2.7	1.1
1.6	4.3	2.7	4.3	2.7	2.7	1.1	2.8	1.2
2.0	3.0	1.0						
				LYMPHOSARCOMA				
3.2	4.8	1.6	4.3	1.1	2.4	—0.8	1.7	—1.5
0.9	3.0	2.1	3.2	2.3	2.0	1.1	2.0	1.1
2.5	4.5	2.0	4.1	1.6	2.0	—0.5	1.7	—0.8
3.7	4.6	0.9	4.4	0.7	0.2	—3.5	0.9	—2.8
3.2	4.9	1.7	4.3	1.1	2.4	—0.8	1.7	—1.5
				RHABDOMYOSARCOMA				
3.1	5.6	2.5	5.0	1.9	4.4	1.3	5.8	2.7
1.1	2.8	1.7	2.5	1.4	2.7	1.6	2.3	1.2
3.6	5.0	1.4	4.3	0.7	3.6	0.0	3.6	0.0
1.0	3.3	2.3	3.2	2.2	3.0	2.0	3.5	2.4
2.2	4.6	2.4			2.2	0.0		
0.7	3.3	2.6			1.0	0.3		
2.9	5.4	2.5			2.9	0.0		
				RAT LEG MUSCLE				
2.1	11.3	9.2	11.8	9.7	5.3	3.2	7.5	5.4
1.4	10.8	9.4	9.2	7.8	5.4	4.0	8.0	6.6
0.8	10.6	9.8	10.5	9.7	6.0	5.2	7.7	6.9
1.3	9.7	8.4	8.6	7.3	4.8	3.5	3.6	2.3
				MOUSE LEG MUSCLE				
1.2	9.7	8.5	9.7	8.5	5.5	4.3	6.6	5.4
0.4	8.3	7.9			4.3	3.9	6.3	5.9
0.9	9.3	8.4	9.7	8.8	5.2	4.3	7.2	6.3

TABLE III

RESPONSE BY V_2 CARCINOMA SLICES TO SUCCINATE (0.018*M*)

Q_{O_2} Succ.	Q_{O_2}	Change, %
7.05	5.65	+23
7.50	6.40	+17.2
4.45	2.80	+58.9
3.60	3.30	+26.9
5.65	4.53	+31.5

RESPONSE BY V_2 CARCINOMA SLICES TO p-$C_6H_4(NH_2)_2$ (0.009*M*)

Q_{O_2} p-$C_6H_4(NH_2)_2$	Q_{O_2}	Change, %
4.85	5.65	—14.2
5.30	5.30	—17.2
3.35	3.20	+ 4.7
4.50	4.71	— 8.90

RESPONSE BY V_2 CARCINOMA HOMOGENATE TO SUCCINATE AND PARAPHENYLENEDIAMINE. CU. MM. OXYGEN UPTAKE PER MGM. DRY WEIGHT PER HOUR

No addition	Succinate 0.018*M*	Δ	p-$C_6H_4(NH_2)_2$	Δ
0.6	1.6	1.0	1.5	0.9
0.7	1.4	0.7	1.5	0.8

This phenomenon may represent real differences in the enzyme systems, when compared with normal homologous tissue slices. However, when dealing with enzymes contained in tissue slices or intact tissue several facts have to be borne in mind. Failure of response to a substrate may mean lack or deficiency of the enzyme that should act on the given substrate, or impermeability of cell membranes to the given substrate. It may also be interpreted as due to the loss of a coenzyme factor, which could diffuse from the tissue into the solution. The permeability of tumor cells is still discussed. Some authors find the permeability increased (9, 17). In tissue culture, the permeability of normal and sarcomatous fibroblasts for water has been found not to differ (1). These facts might imply that the permeability of tumor cells may be changed for some substances while not for others.

The results presented above indicate that homogenates of tumor tissue exhibit a low responsiveness to succinate and paraphenylenediamine. They behave as do the corresponding tumor slices.

Homologous normal tissue homogenates exhibit the definite response mentioned above to both succinate

and paraphenylenediamine, as shown by the slices of the same tissue. These results suggest, therefore, that the response of slices of tumor or of normal tissue to succinate and paraphenylenediamine reflects the cytochrome oxidase system and succinoxidase activities.

As carried out, the test in these experiments yields an indication of the over-all activity of the entire oxidative systems involved:

cytochrome oxidase ⟶ cytochrome *c* ⟶ succinic dehydrogenase ⟶ succinate

The similar behavior of slices and homogenates shows that, in this particular case, the limiting factor is not the impermeability or the diminished permeability to the substrates mentioned above, or the loss, through diffusion in the surrounding medium, of a coenzyme factor.

SUMMARY

Comparisons have been made on the oxidative capacity of a series of homogenates from animal neoplasms and the oxidative capacity of homogenates of normal tissues. The chemical studies included cytochrome oxidase system and succinoxidase activities.

The homogenates behave as do the slices, *i. e.,* tumor homogenates show little or no response either to paraphenylenediamine or to succinate, whereas normal tissue homogenates show considerable response to both substrates.

The results indicate that the low response to paraphenylenediamine and succinate is the same in slices as in homogenates; accordingly, it reflects the over-all activity of the respective oxidative systems involved, and is not due to limiting factors such as permeability.

The oxidative capacity of V_2 carcinoma slices towards succinate and paraphenylenediamine shows that low responsiveness to these two substrates is a property of this neoplasm.

The oxidative capacity of the homogenate from V_2 carcinoma in the presence of the two substrates in question is very low. The homogenate behaves as do the slices.

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Atypical Cell Proliferation in the Anterior Lobe Adenomas of Estradiol-Treated Rats

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It may now be regarded as a generally accepted fact that long-continued treatment with folliculoid compounds such as estrone, estradiol, stilbestrol, etc., causes a definite increase in the size of the anterior lobe in the rat, and may eventually result in the formation of true adenomas. It will not be necessary to survey the pertinent publications here, since the relevant literature has recently been reviewed in some detail (1). The object of this communication is merely to call attention to certain atypical cell types found in the anterior lobe adenomas of rats in which such tumors have been elicited as a result of long-protracted treatment with estradiol.

EXPERIMENTAL

Twenty male albino rats weighing 165 to 180 gm. (average 174 gm.) were subdivided into two groups consisting of 10 animals each. The first group was treated with daily subcutaneous injections of 50 γ of α-estradiol in 0.1 c. of peanut oil; the second received similar daily injections of 50 γ of cholesterol and acted as controls. Cholesterol was chosen for comparison as an agent with which to treat the control group because it has the steroid nucleus in common with the folliculoid hormones yet does not exhibit their specific pharmacological activities.

In both groups treatment was continued for a period of 14 months. At the end of this time all animals were sacrificed. The size of the pituitaries was subject to great variations among the individuals of the estradiol-treated group, since the smallest gland weighed 14 mgm., the largest 189 mgm. Among the cholesterol-treated controls, on the other hand, the weight of the pituitary varied only between 9.3 and 11.8 mgm.

Upon gross inspection the pituitaries of the estradiol-treated animals were found to be very irregular in outline, and exhibited numerous dark red spots. The dark coloration of the greatly distended pituitary cleft was especially obvious. The glands were fixed in "Susa" mixture, embedded in paraffin, and stained by the technic of Selye and McKeown (2). Microscopic examination showed that the red spots, which were visible to the naked eye, consisted of greatly dilated sinusoids, and, in some cases, of blood extravasations in which the erythrocytes were frequently found in process of degeneration. The pituitary cleft always contained a large number of red blood cells, many of which were more or less completely transformed into a basophilic granular material. Within the anterior lobe itself numerous cystic spaces were seen and they appeared to be filled with the same type of basophilic material. In some of these cysts needle-shaped spaces remained light within the granular mass that filled out the lumen. The nature of these spaces could not be determined. They may have been artefacts, due to irregular spreading of the brittle cyst contents, although it is not impossible that during life needle-shaped crystals might have occupied these spaces. In any case, they resembled the so-called cholesterol slits. In the granular mass within the cysts many unusually large basophilic giant cells were found. These were apparently derived from cells that once lined the cysts but had lost contact with their fellows; in the sections they lay free in the cyst lumen. Most of the giant cells contained 3 or 4 nuclei, usually located in the periphery. Some of the largest cells had a diameter of 50 to 60 μ and contained 8 to 10 nuclei. Quite frequently pigment granules, whose color varied between greenish-brown and dark green, were seen in the cytoplasm. Some of these contained free iron, since they gave a positive Prussian blue reaction. In many such cells numerous small vacuoles were found, so that the cytoplasm assumed a spongy appearance (Figs. 1 and 2). In other areas also of the anterior lobe, and especially in cell masses forming partitions between two cavernous spaces, similar giant cells were found, not in cysts but within the anterior lobe tissue itself (Fig. 3). In these locations an unusually large number of anterior lobe cells in the vicinity of the giant cells was in process of mitotic division (Fig. 4). Other basophilic giant cells in these same regions contained one or more orange-colored, and in some cases bright red, fuchsinophilic, crystalloid needles. Amorphous pigment granules were most commonly found in the large giant cells with 4 or more nuclei, while the crystalloids were

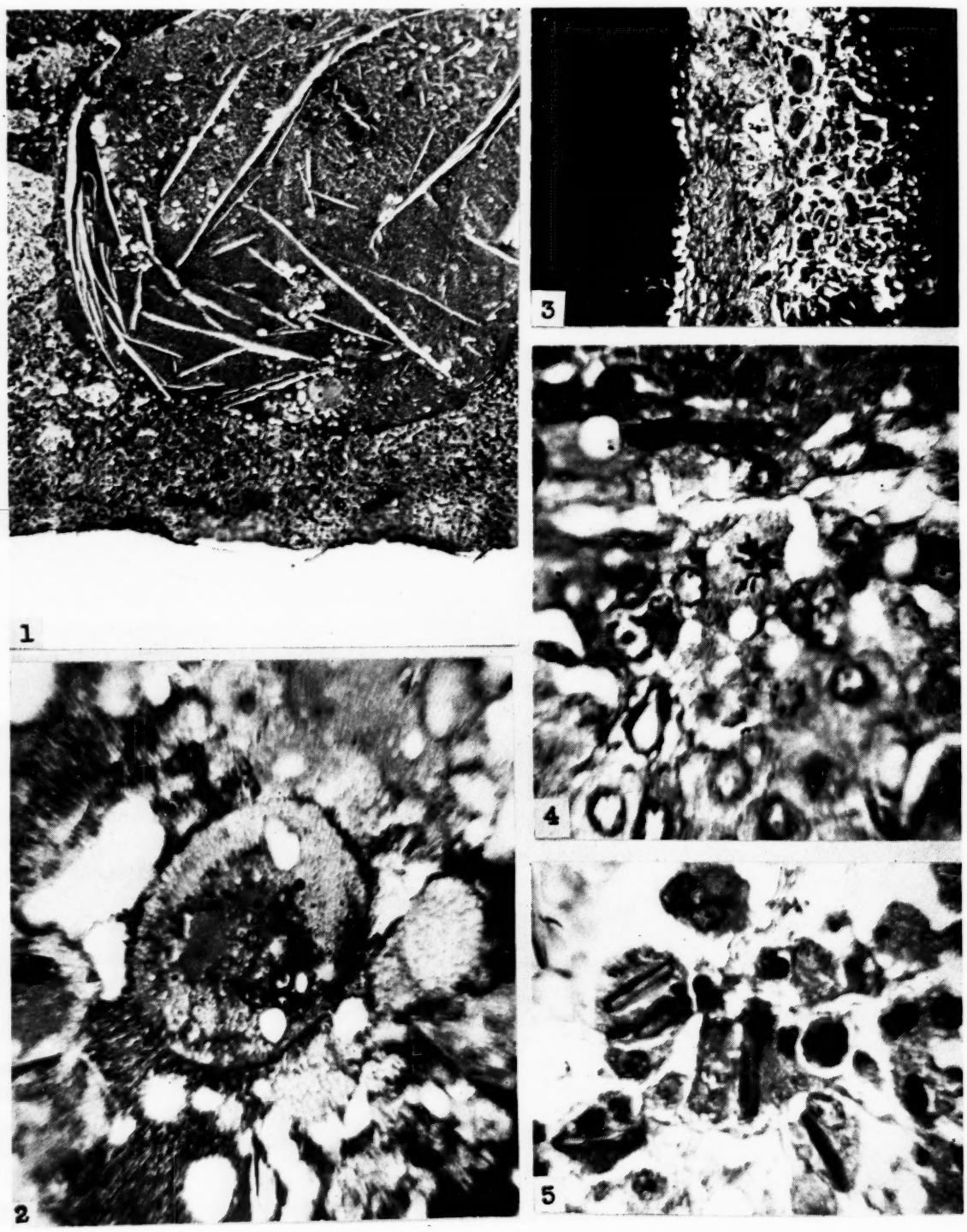

FIG. 1.—Low magnification of a cystic cavity in the adenomatous anterior lobe of an estradiol-treated rat. Note granular material that fills the lumen of the cyst, and light, needle-shapedspaces resembling cholesterol slits, as well as free polynuclear giant cells within the granular mass.

FIG. 2.—High magnification of a giant cell containing 3 distinct nuclei. There are amorphous pigment granules in the center and numerous small vacuoles in the periphery of the cytoplasm.

FIG. 3.—Adenomatous tissue forming a partition between two blood-filled cavernous spaces. Note numerous polynuclear giant cells in it.

FIG. 4.—One of the many mitotic figures seen in the adenomas.

FIG. 5.—Several basophilic cells with crystalloid inclusions.

more common in the smaller giant cells with only 2 or 3 nuclei. In exceptional cases, however, large crystalloid needles measuring up to 25 to 35 μ in length were surrounded by a single polynuclear cell mass.

It should be emphasized that the macroscopic and microscopic appearance of the pituitaries in the α-estradiol-treated group was essentially uniform in spite of the great variation in size. For the sake of brevity we do not discuss each gland separately, but wish to point out that all except the two smallest ones (weighing 14 and 21 mgm. respectively) showed evidence of the atypical cell proliferation described above. It is also noteworthy that under normal conditions mitotic figures are but rarely seen in the anterior lobe of the adult rat, while in the estradiol-treated animals of the present series such figures were unusually abundant. Multinucleated basophilic giant cells such as those described above are never seen in the normal anterior lobe. Since the latter frequently contained crystalloid or pigment inclusions they may be interpreted as an epithelial variety of the foreign-body giant cell type, although endocrine cells are not known to transform themselves into polynuclear phagocytes when in contact with foreign particles. It is also possible that under the influence of continued estradiol action nuclear divisions may proceed too rapidly and hence may not always be followed immediately by cleavage of the cytoplasm. Repeated nuclear divisions of this type would then give rise to the formation of polynuclear cells in whose cytoplasm inclusions may arise either endogenously or through phagocytosis of extracellular particles. The possibility that the giant cells arose secondarily through the fusion of several anterior lobe cells must also be considered but, whatever the mode of their formation may have been, it can be stated with certainty that the giant cells are derived from the specific endocrine epithelial components of the anterior lobe, since intermediate stages between the former and the latter were always demonstrable.

It should be emphasised specifically that even the areas in which the giant cells were most numerous revealed only doubtful evidences of an invasion of the surrounding tissue by the abnormal anterior lobe cells and that metastases of these tumors were never observed.

SUMMARY

The anterior lobe adenomas elicited in rats by long-continued estradiol treatment may exhibit signs of atypical cell proliferation. Such adenomas contained polynuclear giant cells and an unusually large number of mitotic figures. The cytoplasm of the giant cells was basophilic and frequently contained pigment granules or crystalloid inclusions. Metastases or other signs of malignancy were not observed.

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The Heterologous Transplantation of Human Cancers*

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The capacity of some human tumors to survive and to grow in the anterior chambers of the eyes of animals of alien species was reported in a previous paper (1). The failure of later attempts to transplant morphologically benign tumors in this manner suggested that heterotransplantability might be a characteristic property of cancer. Accordingly, a series of experiments was instituted in an attempt to investigate this suggestion and, although the results to date are confirmatory, the group of benign tumors tested is not yet sufficiently inclusive to allow generalization. However, a fairly comprehensive group of cancers has been tested and their successful transplantation appears of sufficient interest to warrant an independent report.

The transfer of 4 human tumors was described in the paper noted above and the present report will be concerned with the primary transplantation of 10 additional cancers. Several of the tumors have been maintained by serial transfer and more detailed studies of their behavior will be presented in later papers.

MATERIALS AND METHODS

The technic of anterior chamber transfer employed in these experiments has been described in detail elsewhere (2).

In all instances the tumor tissue used was obtained by biopsy, and a portion selected for transfer by means of frozen section examination. Selection was based on the degree of cellularity and the absence of necrosis or desmoplastic reaction. As a rule transfer was performed within an hour of operation, but in a number of cases takes were obtained with tissue kept at icebox temperature for 12 hours.

Previous experience had shown the superiority of the guinea pig over the rabbit as a host for human tissue and this species was used exclusively in the present series of experiments.

RESULTS

The results of transfer are presented in summarized form in Table I. The interval between transfer and the appearance of signs of growth refers in all instances to the earliest take in the group. In the great majority of cases growth became apparent in all the successful transplants within a space of several days, and the recorded figure is representative of the group and is of value as an indication of the growth potential of the tumor concerned. It should be noted, however, that the appearance of growth in individual transplants was occasionally delayed for considerable periods of time. Thus in the case of the mammary sarcoma (tumor 7) 4 of the transplants showed vascularization and increase in size on the 60th day, but such signs were not evident in the fifth animal until the 115th day. Variations of this type were not related to the age or sex of the guinea pig and their cause remains obscure.

Fibrosarcoma.—A fibrosarcoma arose in the chest wall of a 63 year old man, and the tissue used for transfer was obtained from the second and third recurrences of the primary growth. The patient died with widespread metastasis 2½ months after the last

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** Fellow of The Jane Coffin Childs Memorial Fund for Medical Research.

DESCRIPTION OF FIGURES 1 TO 4

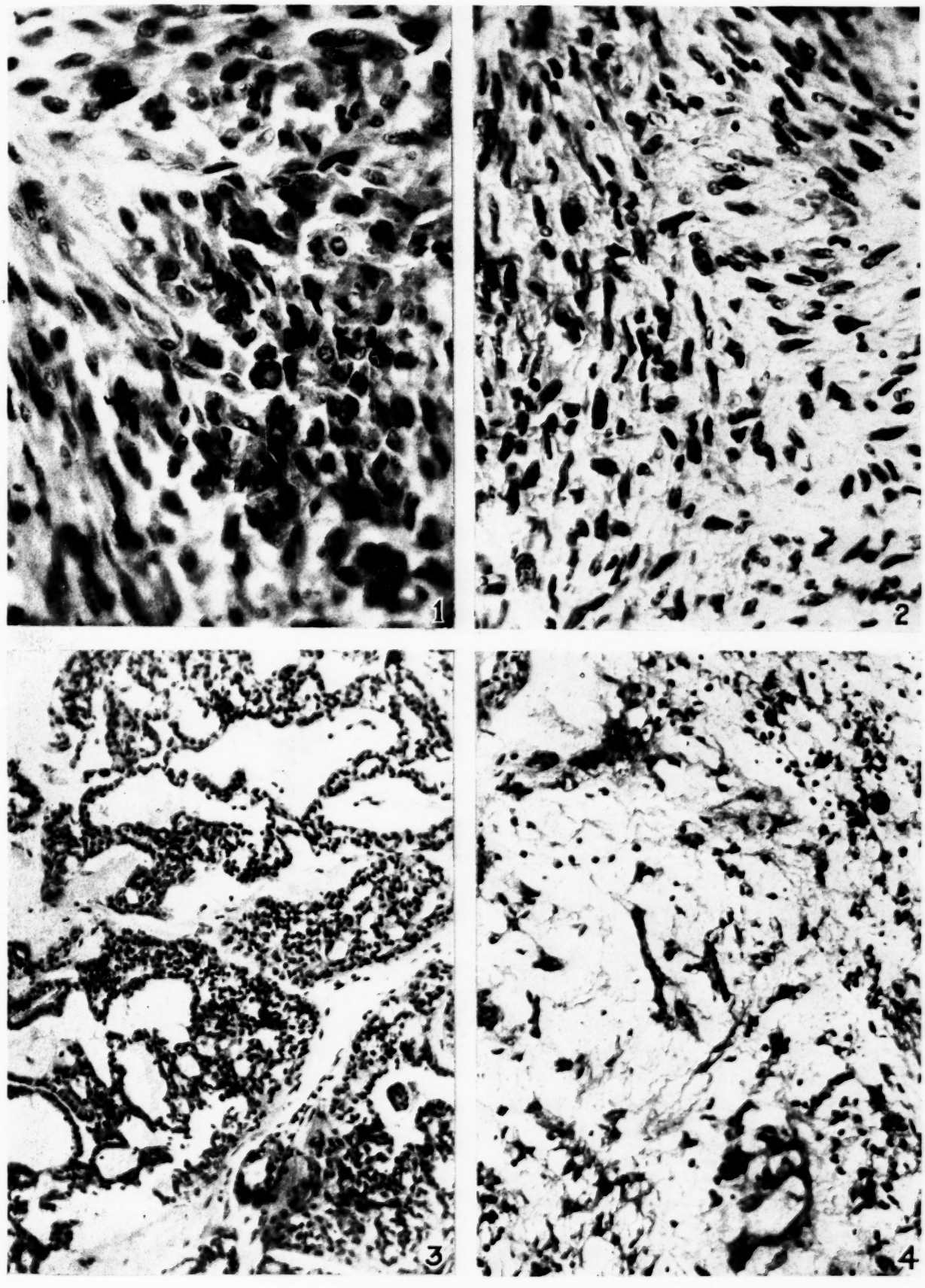
All sections were stained with hematoxylin and eosin.

FIGS 1.—Second recurrence of fibrosarcoma of chest wall (tumor 1). Mag. × 475.

FIG. 2.—Anterior chamber transplant of tumor shown in Fig. 1. The guinea pig bearing transplant was killed 108 days after transfer. Note relatively mature fibroblastic proliferation with absence of giant cells. Mag. × 475.

FIG. 3.—Adenocarcinoma of aberrant salivary gland tissue in the hard palate (tumor 2). Mag. × 220.

FIG. 4.—Anterior chamber transplant of tumor shown in Fig. 3. The guinea pig bearing transplant was killed 150 days after transfer. Note abundant mucinous stromal background with cords of epithelial cells forming abortive glandular structures with a decided resemblance to a mixed salivary gland tumor. Mag. × 235.



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FIGS. 1-4

transfer. Histologically the tumor was made up of fibroblastic elements of sarcomatous character with an unusual amount of giant cell proliferation (Fig. 1).

The first transfer, undertaken on July 14, 1942, gave rise to 2 takes in the 14 animals used. Growth was noted on the 90th day in one instance and on the 194th day in the other and both transplants grew to fill approximately one-quarter of the chamber during a two weeks' period. The first animal was killed for section on the 108th day while the other was held for further observation. In the latter case continued growth was followed by interstitial keratitis with glaucoma, and by the 220th day the corneal opacity so obscured the graft that direct study became impossible. When the animal was killed, on the 275th day, the transplant showed pronounced regressive changes.

The second transfer, 4 months later, resulted in takes in 7 out of 9 animals and growth was evident in all instances on the 21st day. The subsequent course of the transplants was extremely irregular, with phases of apparent reduction in size alternating with periods of renewed growth, and in no instance did the tumor fill the chamber, despite the fact that all the animals were held under observation for 150 days.

Histologically the transplants were similar to the primary tumor. The fibroblastic proliferation was generally of a more mature type with a reduction in the number of mitotic figures and an absence of giant cell forms. Intercellular substance was more abundant (Fig. 2).

Salivary gland adenocarcinoma.—An adenocarcinoma originating in aberrant salivary gland tissue was found in the left posterior portion of the hard palate of a 23 year old man. The patient also presented a number of enlarged, firm lymph nodes in the right submaxillary region. The primary tumor in the palate was removed and transplanted on January 30, 1943, and approximately a month later a right neck dissection was performed, and tissue from the involved nodes also was used for transfer. At the present time, February, 1944, the patient has a mass of large nodes in the left submaxillary region but no further evidence of extension or metastasis is apparent.

Upon microscopic examination the primary tumor

TABLE I: THE RESULTS OF TRANSFER OF 10 HUMAN CANCERS

Tumor No.	Microscopic diagnosis	Date of transfer	Material used for transfer	No. of guinea pigs	No. of takes	From transfer to observable growth, days
1.	Fibrosarcoma	July 14, '42	2nd local recurrence	14	2	90
		Nov. 25, '42	3rd " "	9	7	21
2.	Adenocarcinoma of salivary gland	Jan. 30, '43	Primary tumor	5	3	60
		Feb. 25, '43	Lymph node extension	11	8	20
3.	Chondromyxosarcoma of larynx	Feb. 5, '43	Primary tumor	8	6	13
4.	Malignant melanoma	Mar. 6, '43	Lymph node extension	10	6	23
5.	Epidermoid carcinoma of buccal mucosa	Mar. 31, '43	" " "	9	3	19
6.	Adenoacanthoma of urethra	May 25, '43	" " "	10	1	27
7.	Fibrosarcoma of breast	Aug. 5, '43	Primary tumor	8	5	60
8.	Undifferentiated carcinoma of lung	Sept. 28, '43	Operative implant	7	2	17
9.	Epidermoid carcinoma of lung	Dec. 16, '43	Chest wall extension	6	3	11
10.	Chordoma	July 23, '43	Primary tumor	12	0	
		Oct. 13, '43	" "	6	3	12

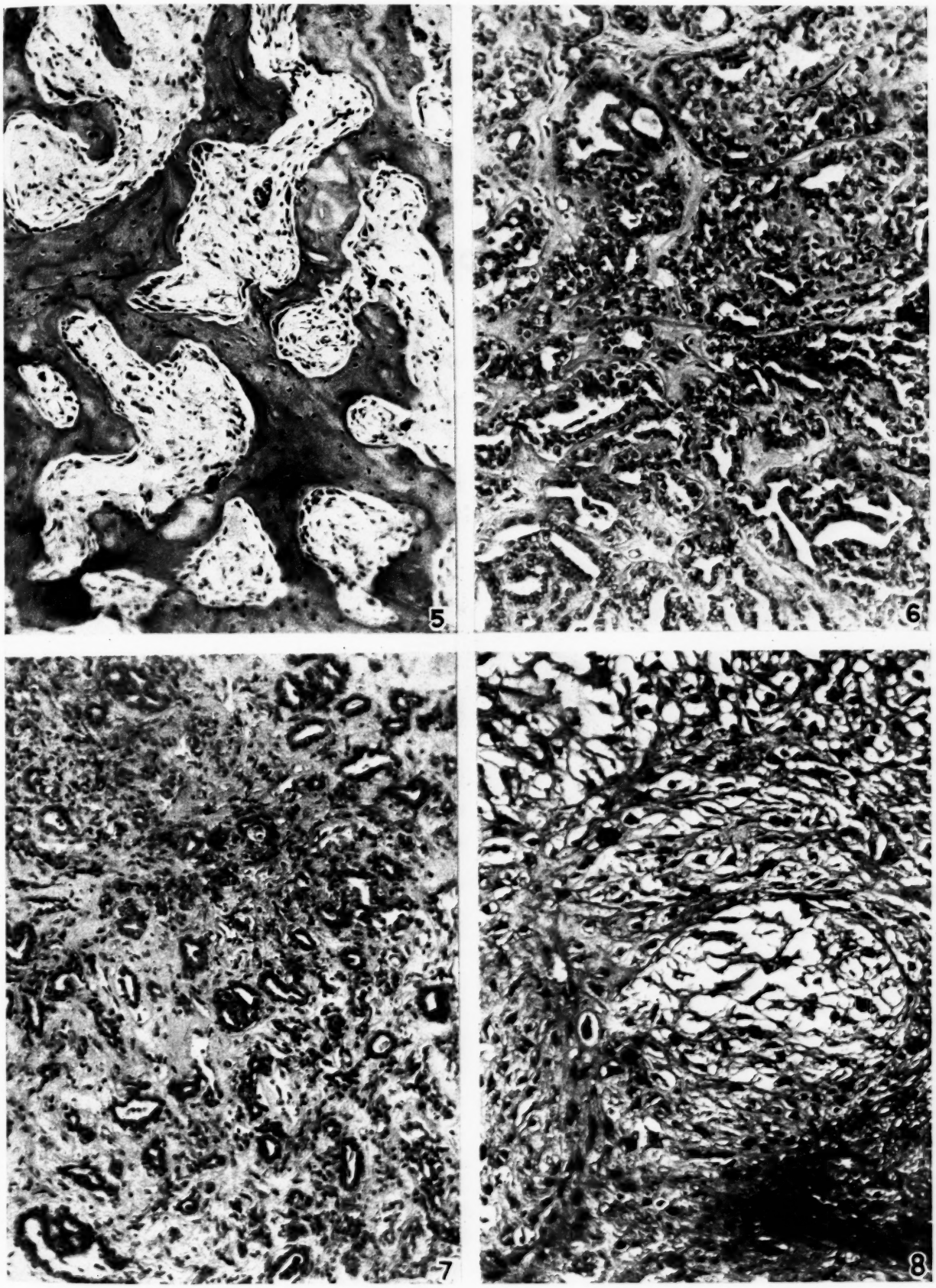
DESCRIPTION OF FIGURES 5 TO 8

FIG. 5.—Anterior chamber transplant of tumor shown in Fig. 3. The guinea pig bearing transplant was killed 150 days following transfer. Fibrous connective tissue, cartilage, and bone. Mag. × 135.

FIG. 6.—Extension of salivary gland adenocarcinoma shown in Fig. 3 to submaxillary lymph nodes. Mag. × 220.

FIG. 7.—Anterior chamber transplant of tumor shown in Fig. 6. Mag. × 220.

FIG. 8.—Chondromyxosarcoma of cricoarytenoid cartilage (tumor 3). Mag. × 230.



FIGS. 5-8

was found to be an adenocarcinoma arising in a "mixed tumor" of aberrant salivary gland tissue (Fig. 3). Growth of the transplanted fragments was evident in 3 of the 5 animals on the 60th day after transfer. The transplants grew slowly and no more than doubled their size by the 100th day. The animals were killed on the 150th day, and at this time all the transplants appeared living and occupied approximately one-quarter of the anterior chamber. Microscopic examination disclosed 2 types of tissue. In some areas epithelial cells formed abortive glandular structures in an abundant mucinous stromal background (Fig. 4); in others, mesoblastic tissues predominated with the formation of cartilage and bone (Fig. 5).

The lymph nodes removed on February 5th were found to be almost completely replaced by epithelial cells in adenocarcinomatous arrangement (Fig. 6). Fragments of this tissue were transferred to the eyes of 11 guinea pigs, and growth was apparent in 8 of these between the 20th and 30th days. The transplants rapidly increased in size to fill approximately one-fifth of the chamber on the 50th day. Thereafter the growth rate decreased, and in several instances no further increase in size occurred despite the fact that the tissues remained alive. The animals were held under observation for varying periods of time up to 102 days.

The transplants in animals killed before the 50th day were microscopically identical with the tissue used for transfer (Fig. 7). However, the transplants in animals killed at later periods showed an entirely different structure, consisting entirely of bone and cartilage similar to that found in transplants of the primary tumor.

Chondromyxosarcoma of larynx.—This unusual tumor arose from the cricoarytenoid cartilage of a 61 year old woman, and the tissue used for transfer was obtained at laryngectomy. The patient is alive at the present time but the tumor has recurred locally.

Histologically the growth was characteristic of tumors of this type and consisted of masses of immature cellular cartilage interspersed with islands of myxomatous tissue (Fig. 8).

Representative tissue was transferred to the anterior chambers of 8 guinea pigs and growth was apparent in 6 of these on the 13th day. The subsequent course of the transplants varied in individual animals. In some, growth was progressive throughout the experiment (122 days) and the fragments grew to occupy one-half of the chamber. In others, a decided reduction in growth rate occurred after the transplants had doubled in diameter (25 days) and only slight increase in size was noted on subsequent examinations.

Histologically the larger, actively growing transplants were identical with the primary tumor (Fig. 9). The smaller, arrested growths, on the other hand, consisted of mature cartilage with extensive areas of bone formation.

Malignant melanoma.—An inguinal lymph node removed from a 44 year old man, 3 years after amputation of a foot bearing a malignant melanoma, was found completely replaced by cells of this tumor. Histologically it consisted of solid masses of melanoblasts with typical nuclear structure and numerous mitoses (Fig. 10).

Representative fragments were transferred to the eyes of 10 guinea pigs, and growth was evident in 6 of the transplants on the 23rd day. Subsequent growth was slow but uniform throughout the group, and in all cases the anterior chamber was one-quarter filled on the 50th day and one-half filled on the 100th day. Histologically the transplants were identical with the primary tumor (Fig. 11).

Epidermoid carcinoma of buccal mucosa.—This tumor originated in the buccal mucosa of a 53 year old woman. The primary growth regressed after irradiation in September, 1942, but in March, 1943, enlarged, firm lymph nodes were found in the neck and on dissection these were found to be diffusely involved by tumor. The patient died with widespread metastases in July of the same year. Microscopically the tumor was a low grade epidermoid carcinoma consisting of well-formed prickle cells with a pronounced tendency to whorl formation and keratin production (Fig. 12).

Fragments of tumor obtained from lymph nodes at the time of the neck dissection were transferred to the eyes of 9 guinea pigs. Takes occurred in 3 of these and the first signs of growth were evident 19 days after transfer. Unfortunately, all the pigs bearing living transplants died during the early course of growth and the period of observation was limited

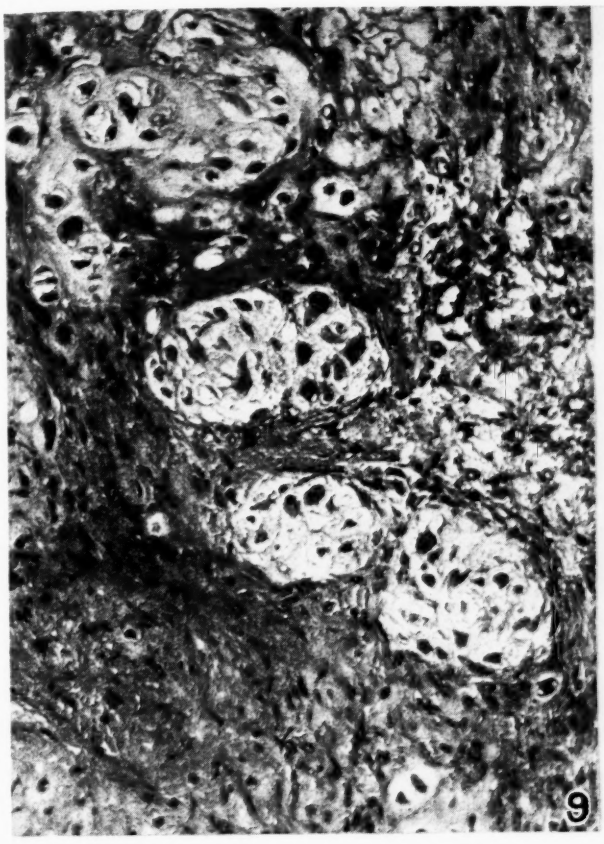
DESCRIPTION OF FIGURES 9 TO 12

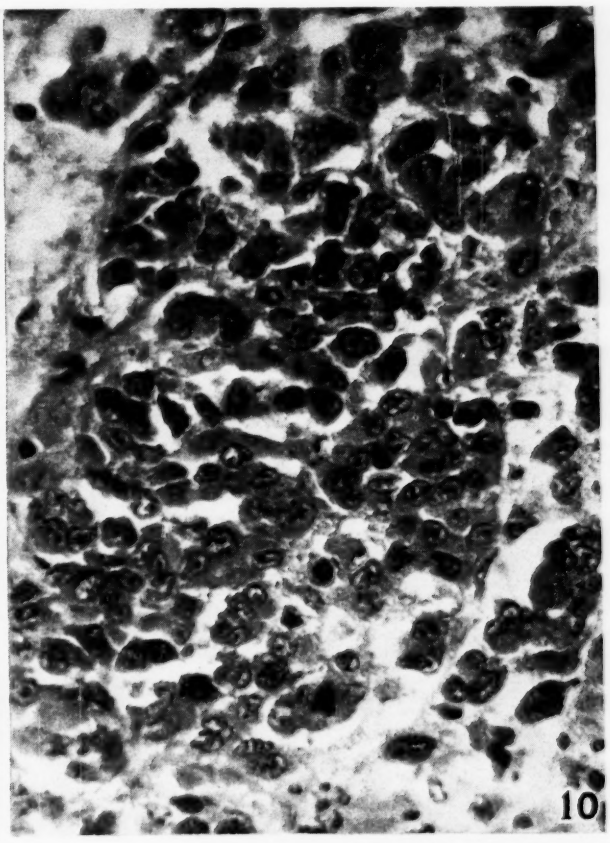
FIG. 9.—Anterior chamber transplant of tumor shown in Fig. 8. The guinea pig was killed 122 days after transfer. Note more mature cartilage and reduction in myxomatous elements. Mag. × 230.

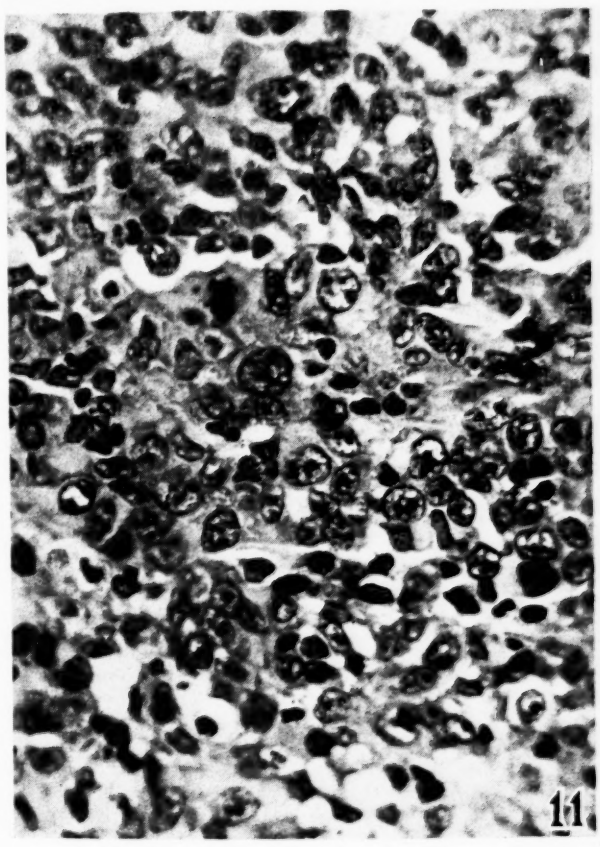
FIG. 10.—Malignant melanoma in inguinal lymph node (tumor 4). Mag. × 575.

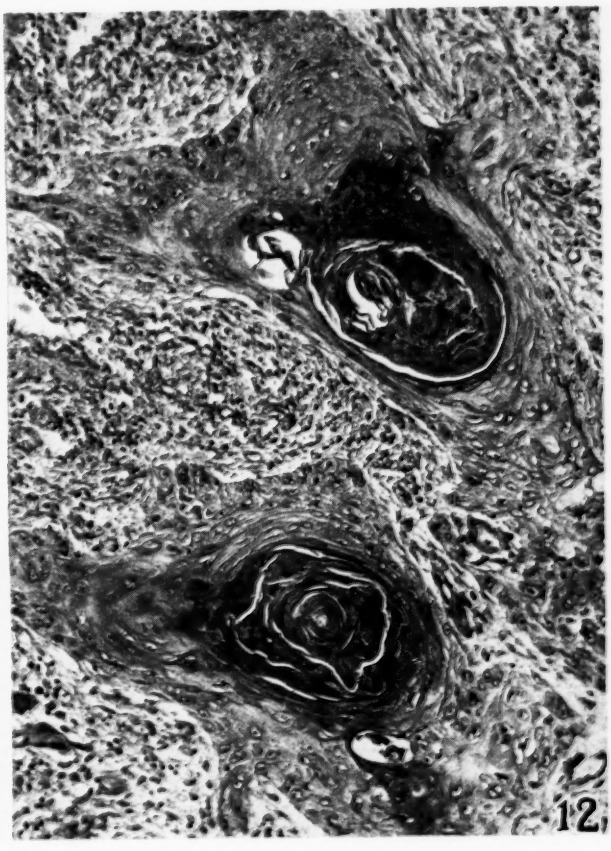
FIG. 11.—Anterior chamber transplant of tumor shown in Fig. 10. Mag. × 575.

FIG. 12.—Extension of epidermoid carcinoma of buccal mucosa (tumor 5) to submaxillary lymph node. Mag. × 165.









FIGS. 9-12

to 33 days. During this period the transplants remained living and grew to occupy one-third to one-half of the chamber. Histologically they were identical with the primary tumor (Fig. 13).

Adenoacanthoma of urethra.—This growth originated in the posterior urethra of a 42 year old man, and the material used for transfer was derived from its extension in an inguinal lymph node. Microscopically the tumor was of some interest inasmuch as the primary growth in the penis was largely epidermoid in character, whereas the lymphatic extension showed a pronounced adenoid architecture (Fig. 14).

The tumor was transferred to the eyes of 10 guinea pigs and a single take resulted. Growth was evident in this instance on the 27th day, and the animal was killed on the 81st day with the anterior chamber two-thirds occupied by tumor. Histologically the transplant was identical with the tumor tissue used for transfer (Figs. 15, 16).

Fibrosarcoma of breast.—A fibrosarcoma arose in the right breast of a 43 year old woman and pulmonary metastases were present at the time of examination. The breast was removed on August 5, 1943, and the patient died 2 months later. Histologically necrosis was a prominent feature of the tumor and the essential neoplastic elements, consisting of fibroblasts with elongated nuclei, were well preserved only in the immediate vicinity of blood vessels (Fig. 17).

Fragments of the tumor were transferred to the eyes of 8 guinea pigs, and takes occurred in 5 instances. Growth was observed in 4 of the fragments on the 60th day, but the fragment in the fifth animal remained unchanged for 115 days. Despite the long latent period the subsequent course of growth was rapid and in this animal, as well as in the others, the transplants grew to occupy two-thirds of the chamber within 2 weeks of the appearance of vascularization. Histologically the transplants were made up of closely packed, large, sarcomatous fibroblasts, and mitotic figures were numerous (Fig. 18).

Undifferentiated carcinoma of lung.—This carcinoma occurred in the left lower lobe of the lung of a 53 year old man. A total left pneumonectomy was performed on August 19, 1943, and on September 28th an implantation growth was removed from the operative site. The patient died on October 10th. Histologically the tumor consisted of solid, unorganized masses of highly anaplastic epithelial cells (Fig. 19).

Fragments of the implantation growth were transferred to the eyes of 7 guinea pigs and takes occurred in 2 instances. Growth was evident on the 17th day in one case and on the 50th day in the other. The anterior chambers of both animals were filled with tumor 2 weeks after the appearance of vascularization. Histologically the transplants were identical with the tissue used for transfer (Fig. 20).

Epidermoid carcinoma of lung.—This tumor arose in the upper lobe of the lung of a 63 year old woman and had invaded the chest wall at the time of operation. Histologically it was found to be made up of irregular masses of squamous cells with numerous mitotic figures and very little keratin formation (Fig. 21).

Fragments of the tumor from the chest wall were transferred to the eyes of 6 guinea pigs, and growth was evident in 3 of these on the 11th day. Subsequent growth was rapid and in all instances the anterior chambers were filled with tumor at the end of a month. Histologically the transplants were identical with the tumor used for transfer (Fig. 22).

Chordoma.—This tumor arose in the region of the 6th cervical vertebra of a 35 year old man. Biopsies were performed on July 23rd and Oct. 13th, 1943, and transfers to guinea pigs' eyes were carried out in both instances. The tissue obtained at the first biopsy was made up of highly anaplastic cells and no classification other than sarcoma was made. Following this biopsy, x-ray therapy was instituted and the patient received a total dose of 1,139 r. Tissue from the second biopsy was largely necrotic but in scattered areas masses of cells were well preserved and their physaliphorous character, together with the nature of the stroma, suggested that the tumor might be a chordoma (Fig. 23).

Tissue from the first biopsy was transferred to the anterior chambers of 12 guinea pigs, and although the animals were held under observation for 5 months no growth occurred. On the other hand, takes occurred in 3 of the 6 animals used in the second transfer. Moreover, growth was apparent in these instances in 10 days and the anterior chambers were one-half filled with tumor by the 40th day, when the animals were killed to obtain tissue for serial trans-

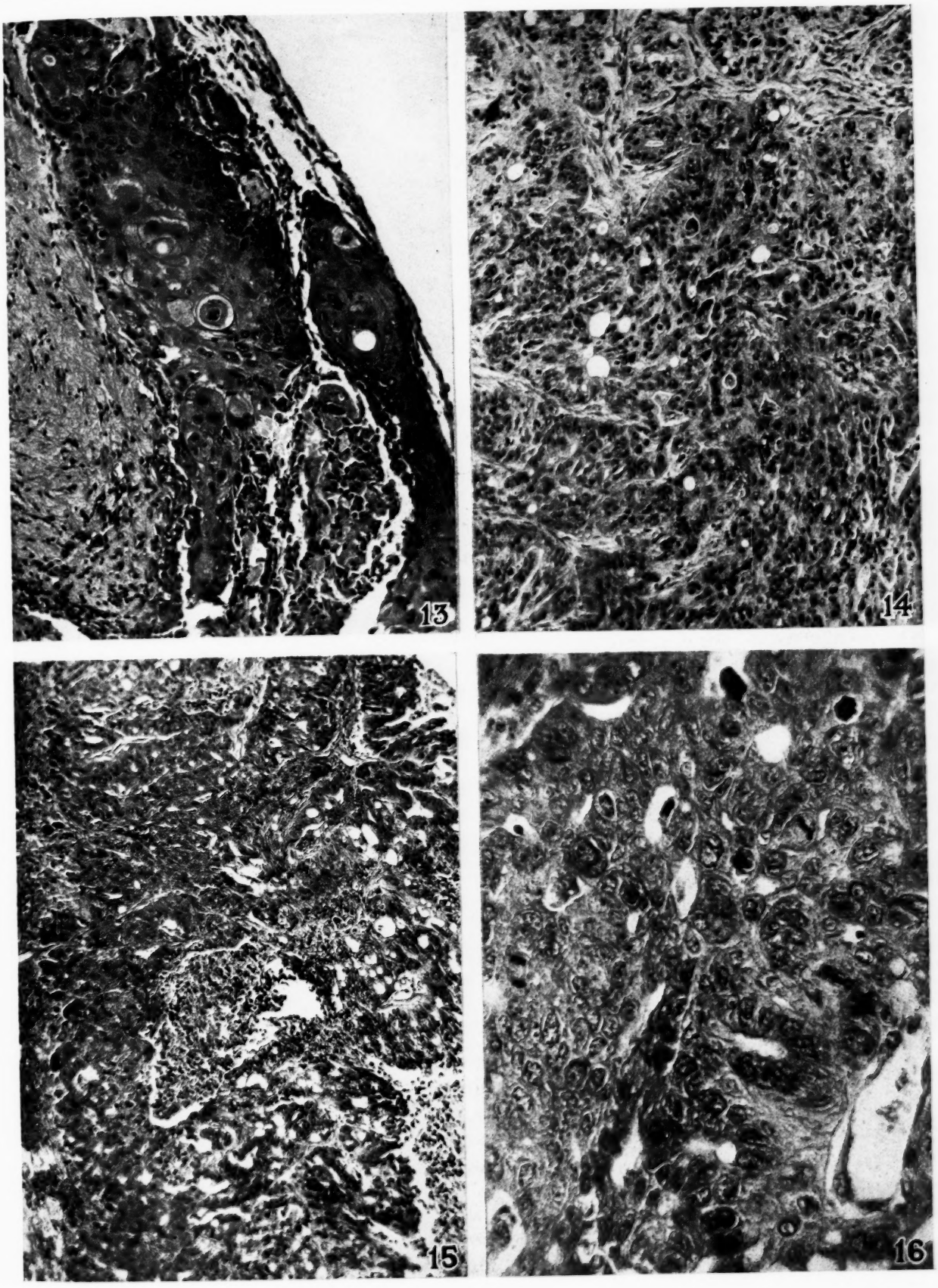
DESCRIPTION OF FIGURES 13 TO 16

FIG. 13.—Anterior chamber transplant of tumor shown in Fig. 12. The guinea pig was killed 33 days after transfer. Mag. × 165.

FIG. 14.—Extension of adenoacanthoma of prostatic urethra (tumor 6) to inguinal lymph node. Mag. × 165.

FIG. 15.—Anterior chamber transplant of tumor shown in Fig. 14. The guinea pig was killed 81 days after transfer. Mag. × 140.

FIG. 16.—Higher power view of transplant shown in Fig. 15. Mag. × 425.



FIGS. 13-16

plantation. Histologically the transplants were found to consist of typical ballooned cells with characteristic vegetable-like limiting membranes and here and there a tendency to the formation of syncytia (Fig. 24).

DISCUSSION

It has been consistently observed in experiments involving rabbit tumors that heterologous transplantation can be successfully effected only when the growth under study has manifested the ability to invade foreign tissue or to metastasize in the primary host. The transfer of benign tumors, or of potentially malignant tumors during their preinvasive stages, invariably fails and the conclusion appears justified that, in the rabbit, autonomy is attained only after continued development and is not a property of the primary neoplastic focus or of other preinvasive stages.

The present series of investigations was instituted in an attempt to determine the validity of these conclusions as applied to human tumors. The experiments reported in this paper are confirmatory as far as concerns the heterologous transplantability of cancer and demonstrate that, in man as well as in the rabbit, cancer autonomy transcends species barriers.

Numerous attempts have been made to transfer benign human tumors to lower animals, but with the exception of several debatable and poorly understood growths of peripheral nerves no takes have been obtained. In addition, a considerable number of transplantation experiments have been performed utilizing biopsy tissue from precancerous lesions and from anaplastic tumors during preinvasive stages, and without exception transfer has been unsuccessful. Such cases are being followed with additional transfers as more advanced biopsy material becomes available, but the performance of serial biopsies on developing tumors is not tolerated by the majority of human patients and the compilation of adequate data is dependent on fortuitous circumstances. However, in several cases of the series reported here tissue from consecutive biopsies of the same tumor was available for transfer, and the results, together with those obtained from the transfer of benign tumors, indicate that in man, as in the rabbit, autonomy is the outcome of continued development and is not a common attribute of all neoplastic cells.

The chordoma (tumor 10, in Table I) is a case in point. Here the first attempt at transfer failed whereas the second transfer, undertaken 2½ months later, gave rise to growth in half of the animals used. The fibrosarcoma (tumor 1) and the salivary adenocarcinoma (tumor 2) are also suggestive in this direction and indicate further that autonomy may be of gradual rather than of sudden development. The first transplantation of the fibrosarcoma resulted in takes in only 2 of 14 animals and growth was not evident until the 90th day, while the second transfer, undertaken 4 months later, gave rise to takes in 7 of 9 animals and growth was apparent on the 21st day. In like manner, transfer of the salivary gland adenocarcinoma gave evidence of a gradient in transplantability or autonomy. The first transfer of this tumor, utilizing the primary growth in the hard palate, resulted in 3 takes in the 5 animals used, and growth was evident on the 60th day. In this case the structure of the primary growth was not reproduced on transfer and the histological appearance of the transplant was that of a mixed salivary gland tumor, presumably an earlier morphological form of the primary growth. On the other hand, the second transfer a month later, utilizing an involved lymph node, resulted in takes in 8 of 11 animals and growth was evident in 20 days. Moreover, the microscopic structure of this tumor and of the transplant were identical.

It was also observed in a study of rabbit tumors that transplantability or autonomy was not immediately related to the degree of anaplasia, and a similar condition appears to hold with reference to human tumors. In the case of the chordoma, tissue obtained at the first biopsy was so highly anaplastic that classification was not possible, while the second biopsy specimen contained sufficiently differentiated cells to suggest the correct diagnosis. In contrast, transfer from the first biopsy failed while transfer from the second was successful. Again, tissue obtained from the 2 operations on the fibrosarcoma of the chest wall showed a comparable degree of anaplasia, yet transfer of tissue from the later biopsy gave rise to a much higher percentage of takes with much earlier growth. The transfers of the 2 lung cancers are also of interest from this point of view. The highly anaplastic tumor (No. 8) grew in 2 of 7 test animals and growth was evident on the 17th day. On the other hand, the more differentiated epidermoid tumor (No. 9)

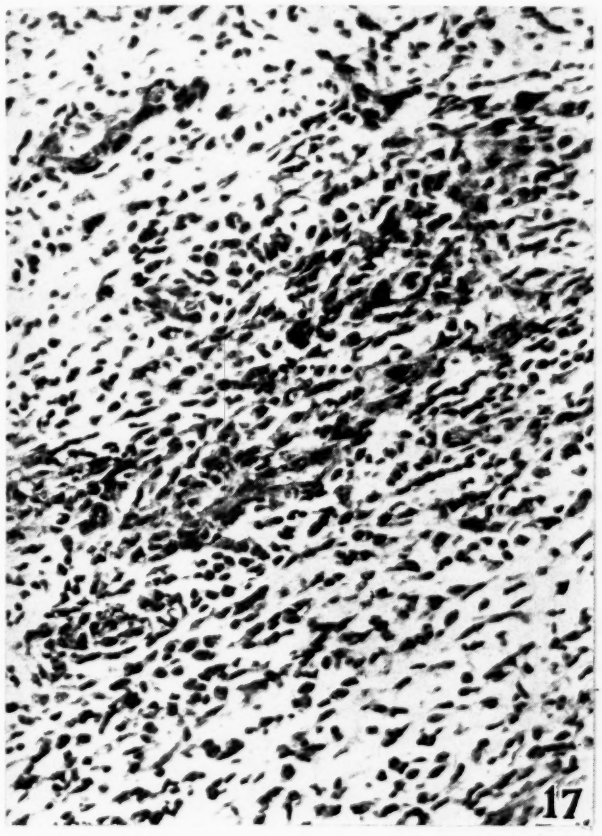
DESCRIPTION OF FIGURES 17 TO 20

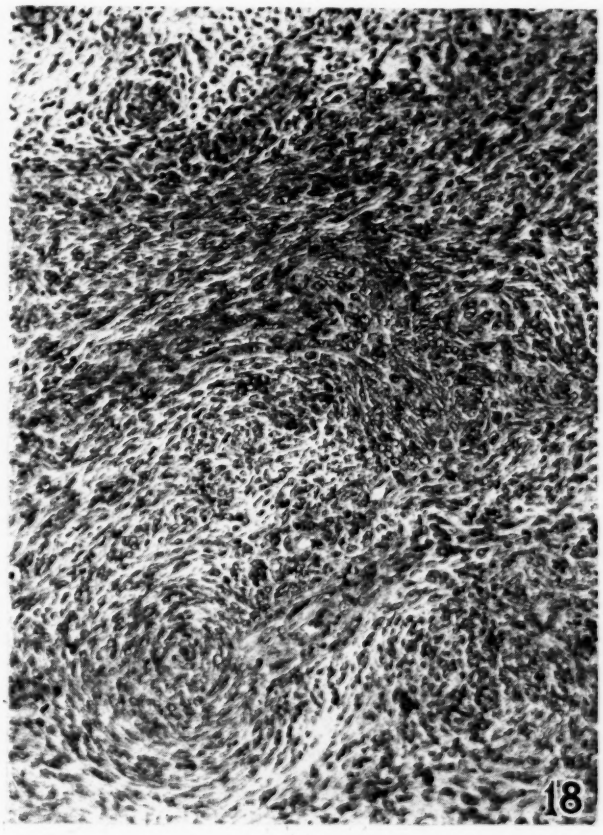
FIG. 17.—Fibrosarcoma of human, female breast (tumor 7). Mag. × 165.

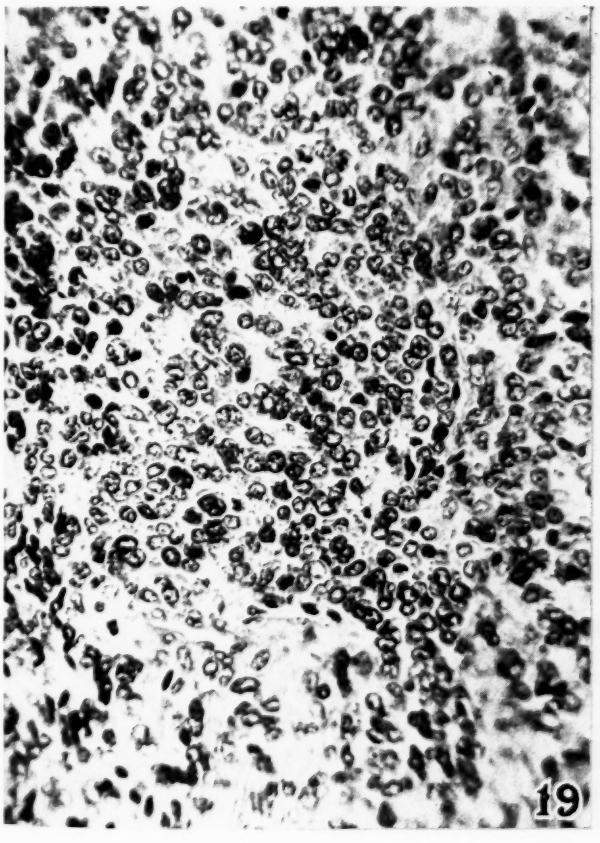
FIG. 18.—Anterior chamber transplant of tumor shown in Fig. 17. The guinea pig was killed 78 days after transfer. Mag. × 165.

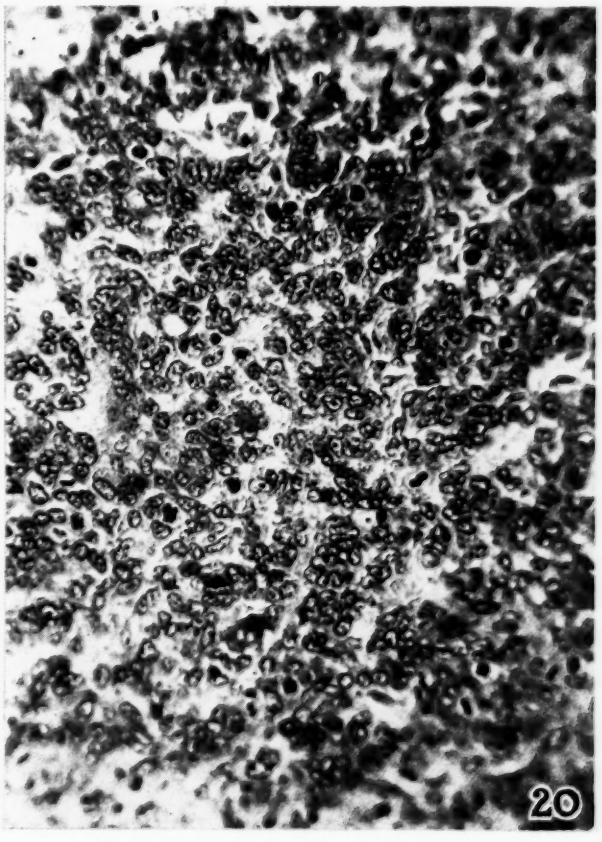
FIG. 19.—Operative implant from primary undifferentiated carcinoma of lung (tumor 8). Mag. × 250.

FIG. 20.—Anterior chamber transplant of tumor shown in Fig. 19. The guinea pig was killed 64 days after transfer. Mag. × 250.









FIGS. 17-20

grew in 3 of 6 animals and growth was apparent by the 11th day. A study of the other tumors of this series fails to show the existence of a correlative relationship between autonomy and the degree of differentiation, a finding that is of some significance in view of present attempts in surgical pathology to offer prognoses based on grades of anaplasia.

Transfer to lower animals is not only a diagnostic aid in the case of questionable tissues but also assists in the morphological classification of cancers. Occasionally cancers are associated with a local tissue reaction that obscures the nature of the cell involved in the neoplastic process. Thus the tissue obtained from the first biopsy of the fibrosarcoma of the chest wall contained a large number of giant cell forms, and a question of muscular origin arose. However, only the fibroblastic elements survived guinea pig transfer, and it became clear that the growth was a fibrosarcoma and that the giant cells were reactive rather than neoplastic in nature. Fortunately, also, transplants often show a slightly higher degree of cellular differentiation and organization than is found in the primary host and thus allow a classification of highly anaplastic tumors. For example, the tissue from the vertebral growth (tumor 10) sent to the laboratory for diagnosis was 90 per cent necrotic and tumor cells were scattered and highly anaplastic. A diagnosis of chordoma was made on the basis of a "hunch" rather than from objective morphological considerations—and widely different views were held by other observers. However, the type and arrangement of cells in the guinea pig transplant left no doubt of the true nature of the growth and substantiated the diagnosis of chordoma.

The ability to grow cancer in lower animals affords an approach to many other problems associated with human tumors. After successful primary transplantation the cancer can be carried by serial passage to new generations of animals and subjected to a variety of investigations not permissible during residence in the human host. It should be emphasized in this connection that after preliminary growth in the anterior chamber transfer to other body regions is readily effected.

SUMMARY

A series of 10 human cancers including a fibrosarcoma of the chest wall, an adenocarcinoma of salivary gland tissue, a chondromyxosarcoma of the larynx, a malignant melanoma, an epidermoid carcinoma of buccal mucosa, an adenoacanthoma of the urethra, a mammary fibrosarcoma, an undifferentiated carcinoma of the lung, an epidermoid carcinoma of the lung, and a chordoma have been successfully transferred to the anterior chambers of the eyes of guinea pigs. The transplants grow progressively in the alien host and bear a close histological resemblance to the original tumors.

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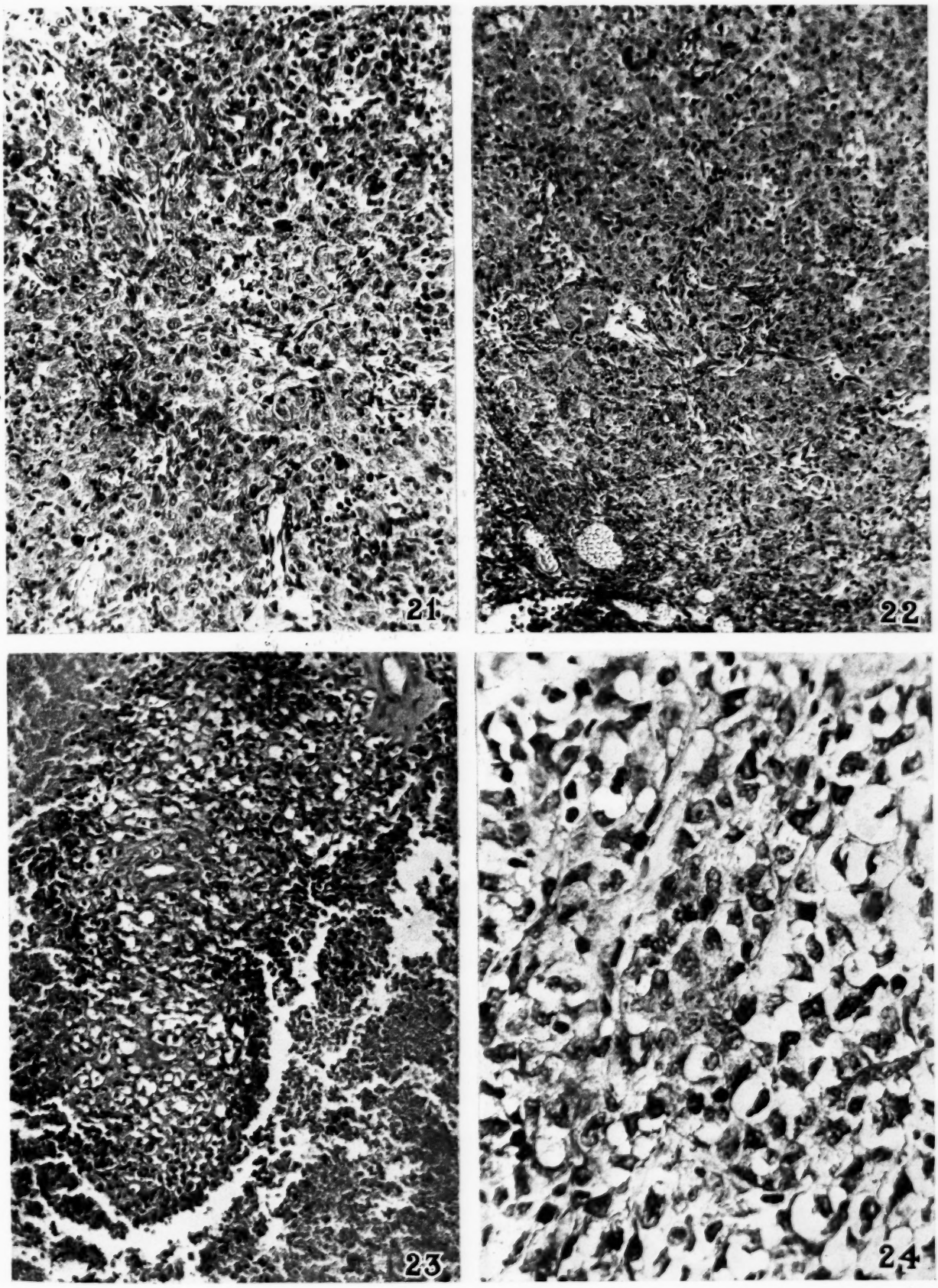
DESCRIPTION OF FIGURES 21 TO 24

Fig. 21.—Pleural extension from primary undifferentiated carcinoma of lung (tumor 9). Mag. × 165.

Fig. 22.—Anterior chamber transplant of tumor shown in Fig. 21. The guinea pig was killed on the 31st day after transfer. Mag. × 165.

Fig. 23.—Chordoma arising in region of 6th cervical vertebra (tumor 10). Note extreme anaplasia and extensive necrosis. Mag. × 165.

Fig. 24.—Anterior chamber transplant of tumor shown in Fig. 23. The guinea pig was killed 40 days after transfer. Note physaliphorous character of more highly differentiated cells. Mag. × 575.



FIGS. 21-24

Immunity Reactions Obtained with a Transmissible Fowl Tumor (Olson)

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It has long been known that the regression of an implanted tumor imparts increased resistance or immunity to a second implantation of homologous tumor tissue (11, 12, 13). Autovaccination by the implantation of small amounts of neoplastic tissue into the dermal or subcutaneous tissues has been used by several investigators (1, 2, 4, 6, 7) to study the immunity produced by a regressing tumor. Similar phenomena have been observed by us in experiments with the neoplasm described by Olson (9) as a transmissible lymphoid tumor of the chicken. During the first 30 passages, Olson (9) obtained a tumor incidence of 67.7 per cent when birds were inoculated by the subcutaneous or intramuscular route. Spontaneous regression occurred in 44.3 per cent of those that developed tumors. Grossly visible metastasis to other tissues was observed in 17.0 per cent of the positive birds.

MATERIALS AND METHODS

Pedigreed white leghorn chickens of the Laboratory stock were used in all experiments reported here. The first inoculation was made at ages varying from 36 to 192 days; this variation did not seem to have any influence on results obtained.

The tumor was obtained at the 138th serial passage through the courtesy of Dr. Carl Olson, Jr., and has been under study at this Laboratory during 57 additional transfers. During this time the tumor has been characterized in serial passage by its energetic growth, as evidenced by the rapid rate at which serial transfers can be made (7 days), and by its easy adaptation, as shown by the incidence of takes, which approached 100 per cent. Its malignancy is indicated by early and widespread metastasis, which occurred in 90 per cent of the hosts that died or were killed as donors.

Serial transfers were made at 7 day intervals with 0.5 cc. of minced tumor (10), diluted with an equal volume of Tyrode's solution and injected deeply into the pectoral muscle. Inoculum was obtained from birds in serial transfer and consisted of (a) minced tumor, prepared as described above, and (b) tumor cell suspension, which was prepared by passing the tumor through a mincer, suspending the mince in 2 parts of Tyrode's solution, and filtering through sterile cotton. The number of neoplastic cells was adjusted to 20,000 per $mm.^3$ by first determining the cell concentration in a standard blood cell counting chamber and then making the necessary dilution with Tyrode's solution. The following routes of inoculation were used: intramuscular, intradermal, subcutaneous, and into the septum of the wattle.

The pectoral tumor size was recorded numerically by arbitrarily assigning numbers of 1 to 5 to tumors of increasing size. An average of 3 or 4 readings made at intervals during progressive enlargement was used as the size index.

At the time of passage the tumor consisted of a large mass of neoplastic tissue containing centrally placed and/or miliary foci of necrotic tissue. In preparing the inoculum attempts were made to minimize the amount of necrotic and normal tissue introduced, in order to secure uniformity in the material.

A measure of the size of cutaneous and subcutaneous tumors was obtained by multiplying the maximum diameter in millimeters by the height in millimeters. The mean of 3 or 4 such measurements taken at the time of progressive growth was used as the size index.

RESULTS

In a group of 19 different inoculations (serial transfer and viability studies) tumors appeared in 162 out of 168 birds inoculated with 1.0 cc. of minced tumor. Of the 162 positive birds, 66 died, 55 were killed as donors, and the remaining 46 survived and showed complete regression of the tumor. When the surviving birds were reinoculated at 34 to 202 days after the first implantation, neoplastic growth did not appear in any of the birds, whereas it produced large tumors in all 16 control birds.

Inoculation of immune birds resulted in a transient local vascular reaction. The incidence and extent of this reaction were variable.

In order to determine if these birds were also immune to inoculation by other routes, 0.01 to 0.02 cc. of tumor cell suspension was injected into the dermal layer 55 to 64 days after the second intramuscular inoculation. No skin tumors developed in any birds previously inoculated, whereas all 9 control birds developed tumors.

The results of an experiment to demonstrate the influence of route and dose upon the development of immunity are given in Table I. Birds inoculated intramuscularly with 10,000 to 10,000,000 tumor cells developed tumors that subsequently regressed. Reimplantation of 10,000,000 cells in the opposite muscle 38 days later produced no evidence of tumor formation. Intradermic inoculation of 400,000 cells 48 days after the second inoculation was unsuccessful in producing a growth, whereas tumors appeared in all control birds in which the inoculum was tested for potency.

The intradermic injection of 2,000 to 200,000 neoplastic cells produced local tumors in all birds. The tumors varied in size from 14 to 19 mm. in diameter and 3 to 5 mm. in height. After an interval of 38 days the same birds were reinoculated by the same route, but in another area, with the result that no tumors developed. A third inoculation was made into the pectoral muscle with 10,000,000 cells after a period of 48 days, and all birds were found to be immune.

Injection of viable tumor cells into the wattle or the subcutaneous tissues, or their introduction into the skin by scarification with a hypodermic needle, caused the formation of local tumors that regressed and resulted in immunity to an intradermal implantation 65 days later.

Samples of minced tumors from 7 donors were frozen slowly and stored at −65° to −76° C. for 245 to 384 days (5). Results given in Table II indicate that all but two samples showed a high potency of tumor induction. Samples III and VI produced takes in only 3 of 7 and 2 of 7 birds respectively. All birds were reinoculated with fresh tumor 30 days later and all frozen samples were found to produce immunity except samples III and VI, which failed

TABLE I: THE EFFECT OF ROUTE AND NUMBER OF TUMOR CELLS INJECTED UPON THE RESPONSE TO SUBSEQUENT INOCULATIONS

First inoculation				Second inoculation (38 days after the first)				Third inoculation (48 days after the second)			
Route	No. cells	P/I *	Tumor size index (av.)	Route	No. cells	P/I *	Tumor size index (av.)	Route	No. cells	P/I *	Tumor size index (av.)
Intramuscular	1×10^7	4/4	4.0	Intramuscular	1×10^7	0/4	0	Intradermic	4×10^5	0/4	0
"	2×10^5	3/3	3.2	"	1×10^7	0/3	0	"	4×10^5	0/2	0
"	1×10^4	4/5	2.2	"	1×10^7	0/5	0	"	4×10^5	0/5	0
				"	1×10^7	3/3 †	2.3	"	4×10^5	0/3	0
								"	4×10^5	4/4 †	94
Intradermic	2×10^5	4/4	89	Intradermic	2×10^5	0/4	0	Intramuscular	1×10^7	0/4	0
"	2×10^4	4/4	71	"	2×10^5	0/4	0	"	1×10^7	0/4	0
"	2×10^3	5/5	44	"	2×10^5	0/4	0	"	1×10^7	0/3	0
				"	2×10^5	3/3 †	28	"	1×10^7	0/3	0
								"	1×10^7	3/3 †	5
				(65 days after the first)							
Wattle	1×10^7	3/4	8X	Intradermic	7×10^5	0/4	0				
"	2×10^6	4/4	10X	"	7×10^5	0/4	0				
"	1×10^6	1/3	4X	"	7×10^5	0/3	0				
Subcutaneous	1×10^6	4/4	137	"	7×10^5	0/4	0				
Dermis scarified		2/2	101	"	7×10^5	0/2	0				
				"	7×10^5	4/4 †	86				

* P—number positive, I—number inoculated.
† —control.

TABLE II: EFFECT OF SLOW FREEZING AND STORAGE (245 TO 384 DAYS) UPON THE IMMUNOGENIC PROPERTIES OF MINCED TUMOR GIVEN INTRAMUSCULARLY

		Tumor slowly frozen and stored		Fresh tumor injected 30 days later	
Donor No.	No. birds	No. pos.	Tumor size index (av.)	No. pos.	Tumor size index (av.)
I	8	8	2.9	0	0
II	6	6	4.5	0	0
III	7	3	2.3	6	4.1
IV	7	7	4.7	0	0
V	7	6	3.5	0	0
VI	7	2	2.0	4	4.0
VII	6	6	3.5	0	0
Control	6	—	—	6	3.7

to produce good tumor growth at the first inoculation.

In another experiment minced tumor was frozen rapidly by immersion in a mixture of solid CO_2 and 95 per cent ethyl alcohol and then thawed in tap water. The freezing and thawing were repeated once and then the mixture was injected into the pectoral muscle. The result was that tumors did not appear in any of the birds (Table III). When the same birds were reinoculated 21 days later with fresh tumor inoculum, large growths appeared in all birds. Thus, when inoculations with inactivated tumor mince were made, no immunity resulted.

TABLE III: EFFECT OF RAPID FREEZING UPON IMMUNOGENIC PROPERTIES OF MINCED TUMOR GIVEN INTRAMUSCULARLY

		Inactivated tumor frozen rapidly twice		Fresh tumor injected 21 days later	
	No. birds	No. pos.	Tumor size index (av.)	No. pos.	Tumor size index (av.)
Experimental	21	0	0	21	4.1
Control on 2nd inoculation	10	0	0	10	4.5

DISCUSSION

Large doses (1.0 cc.) of minced tumor injected into the pectoral muscle produced a high proportion of takes with high mortality, whereas smaller doses (tumor cell suspension) produced a similar high proportion of takes but the mortality was reduced to a very low level, thus providing time for regression of the tumor. Inoculation of neoplastic cells by other routes also resulted in a large number of takes, with subsequent regression. Birds that survived the first tumor implantation did not develop tumors upon subsequent implantations.

The extent of the immunity induced by this process is indicated by the following observations:

1. All birds in which an implanted tumor had regressed were found to be immune to a second and third implantation of the same tumor.

2. Birds implanted with tumor cells by any one of several routes were found to be immune to a second implantation by other routes.

3. The period of immunity is reasonably long (at least 202 days).

4. Immunity induced by a small number of neoplastic cells cannot be overcome by subsequent inoculation with as many as 10,000 times the number used in the first inoculation.

These characteristics are at variance with the immune reactions described for other fowl and mammalian transplantable tumors, where it has generally been found (a) that the induced immunity is not obtained in all animals, (b) that it can easily be overwhelmed by large doses of test inoculum, and (c) that the period of immunity is of comparatively short duration.

When tumor mince was frozen rapidly or stored under certain conditions (5) there was a complete or partial loss of its ability to incite tumors or to establish immunity to inoculations of the active agent. These results are in agreement with previous reports (1, 3, 8) showing that active growth and regression of the primary tumor is a necessary adjunct to immunity or increased resistance to subsequent tumor growth.

SUMMARY

The transmissible fowl tumor (Olson), when implanted in a total of 312 chickens by various routes and in appropriate dosages, produced a high incidence of local tumors that upon regression rendered the birds immune to subsequent implantation of the same tumor strain. Immunity obtained with this tumor is of particular interest because (a) it appears in all birds that have survived an active growth of the tumor, (b) it apparently cannot be overwhelmed by very large or repeated doses of the same agent, and (c) it is present over a prolonged experimental period.

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The Effect of Aromatic Compounds upon the Ascorbic Acid Content of the Liver in Mice*

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CONTENTS

The observation that some aromatic hydrocarbons (naphthalene, phenanthrene, anthracene, 3,4-benzpyrene, cholanthrene, methylcholanthrene) undergo aerobic oxidation in the presence of ascorbic acid *in vitro* (12) suggested the experiments *in vivo* described below.

The only investigation on this subject that has been found in the literature is that of Boyland and Mawson (1), who gave single intraperitoneal injections of 3,4,5,6-dibenzcarbazole in olive oil to mixed-stock mice in doses of 0.5 mgm., which caused a mortality of nearly 75 per cent in the first 3 days, or of 0.25 mgm. (57 per cent mortality in 39 days). They found that the amounts of ascorbic acid in the liver were almost wholly within the wide range (152 to 465 γ per gm.) normally found in these mice, while the glutathione content was generally raised, in some cases to twice the normal amount. They carried out comparative experiments with two compounds that do not act especially upon the liver; namely, with methylcholanthrene (1 mgm. in lard per 20 gm. mouse body weight) and 1,2,5,6-dibenzanthracene (2 mgm. in olive oil). Thus their doses were 10 to 20 times smaller than those used in the work described here (Table IV).

METHODS

The compounds tested may be classified as follows:

1. Carcinogenic hydrocarbons; 3,4-benzpyrene, 1,2,5,6-dibenzanthracene, 1,2,5,6-dibenzphenanthrene, cholanthrene, methylcholanthrene, 9,10-dimethyl-1,2-benzanthracene, and the weakly carcinogenic 1,2-benzanthracene.

Table I: Ascorbic Acid in Liver (Boyland and Mawson)

	Number of mice	Days after injection		γ Ascorbic acid per gram liver	
Normal range in mixed-stock mice	—	—		152 to 465	
Intraperitoneal injection					
3,4,5,6-Dibenzcarbazole 0.5 mgm.	21	2 to 17	Mean	235	
" " 0.25 mgm. per 20 gm. body weight	15	10 to 176	"	355	
Methylcholanthrene 1 mgm.	8	4 to 8	"	317	357
" " "	12	12 to 20	"	384	
1,2,5,6-Dibenzanthracene 2 mgm.	8	3 to 10	"	275	

2. Carcinogenic compounds acting chiefly upon the liver; dimethylaminoazobenzene, 2,2′-azonaphthalene.

3. An estrogenic carcinogen; stilbestrol.

4. Noncarcinogenic hydrocarbons; naphthalene, phenanthrene, anthracene.

These were injected in amounts from 1 to 40 mgm., in 0.1 to 0.25 cc. sesame or arachis oil, either subcutaneously, or intraperitoneally, into mice (CBA and MRC male and female, stock male) that were killed

* Because of the difficulties of international communication the authors have not read proof of this article.

from 2 to 19 days later. Controls received injections of solvent only. In all, 700 mice were used.

Estimations of ascorbic acid were carried out in two ways:

1. The liver of a single mouse was ground with 3 per cent metaphosphoric acid (28 cc. in all) and the extract titrated with 2,6-dichlorophenolindophenol; for details see (8). All the experiments with 3,4-benzpyrene recorded in Tables II and III, and some of those with dimethylaminoazobenzene and anthracene (Tables V and VI), were performed in this way.

2. The livers of from 3 to 10 mice were pooled and ground up together either in 3 per cent metaphosphoric acid (35 to 100 cc.) or in similar amounts of $M/15$ KH_2PO_4 (pH 4.7; in this case the extract was precipitated with 1/10 volume 3 per cent metaphosphoric acid, and centrifuged, before titration) in order to obtain a larger volume of extract for comparative titrations by the potentiometric method; this procedure gives less complete extraction, but the same number of control livers was treated in exactly the same way, hence the comparative results should be reliable.

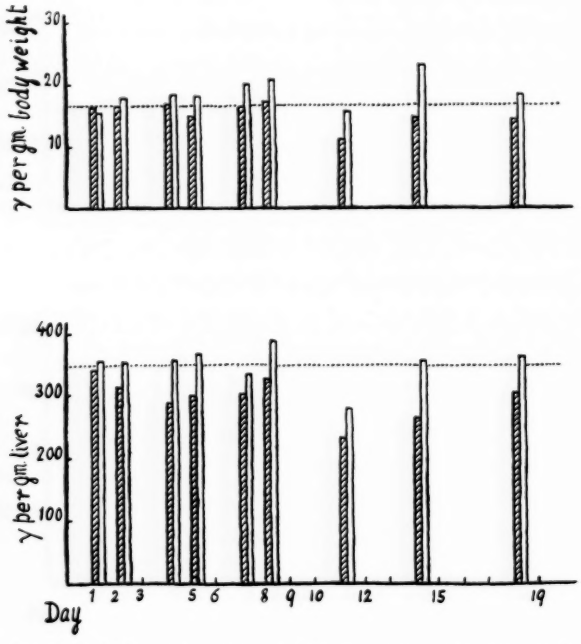

FIG. 1.—CBA male mice. 3,4-Benzpyrene, subcutaneously in sesame oil. In Figs. 1 to 4, hatched columns represent control mice receiving oil only; plain columns represent mice receiving hydrocarbons in oil. In Figs. 1 and 2, horizontal dotted lines represent mean values found in CBA male mice receiving no injections at all (8).

3,4-Benzpyrene

The mean results summarized in Tables II and III, and Figs. 1 and 2, which were obtained from extracts in 3 per cent metaphosphoric acid of livers from 65 individual mice without pooling, show that the injection of 3,4-benzpyrene in sesame oil, either subcutaneously or intraperitoneally, is followed by an increase of ascorbic acid in the liver. In establishing the occurrence of any such increase one encounters the difficulty that the normal range of variation in this amount is of the order of 100 per cent. Thus in 20 normal mice of the kind (CBA males) used in this case the range was from 236 to 436 γ ascorbic acid per gram liver, mean 349 γ (8). Hence each of the batches of mice exposed during various intervals to different amounts of hydrocarbon was accompanied by its own batch of controls, which were similar in age and weight and received injections of the same volume of solvent.

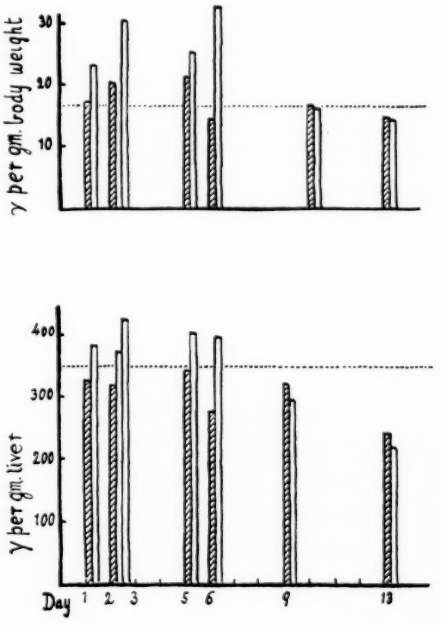

FIG. 2.—CBA male mice. 3,4-Benzpyrene, intraperitoneally in sesame oil.

The amounts per gram liver in the series of subcutaneous injections are actually higher throughout (second to 19th day) in the treated mice, the largest difference appearing on the 15th day (36 per cent increase), and on this day there is the largest increase (62 per cent) in ascorbic acid per gram body weight. In the intraperitoneal series the change is more transient, owing no doubt to more rapid absorption, and has disappeared by the ninth day; the highest figure was obtained (from a single mouse) on the sixth day, when the concentration was 42 per cent more than in the controls, and the amount per gram body weight in the liver, which was enlarged, was greater by 127 per cent.

Seven other experiments, of which two are recorded in Table VII, upon extracts in 3 per cent metaphosphoric acid or $M/15$ KH_2PO_4 of pooled livers of 37 mice in all receiving 20 to 25 mgm. 3,4-benzpyrene in

0.25 cc. arachis oil intraperitoneally 3 days before, and 38 controls, gave confirmatory evidence of a considerable increase in the ascorbic acid of the liver after injection of the hydrocarbon.

Sections of the livers of all the 65 mice referred to in Tables II and III were examined microscopically, obtained by potentiometric titration of extracts in 3 per cent metaphosphoric acid or $M/15$ KH_2PO_4 of the pooled livers of from 3 to 6 mice that had received intraperitoneal injections in 0.25 cc. arachis oil 2 to 7 days previously.

The concentration of ascorbic acid in the liver is

TABLE II: 3,4-BENZPYRENE SUBCUTANEOUSLY IN SESAME OIL

Number of mice (CBA ♂)	Dose of 3,4-benzpyrene, mgm.	Interval after injection, days	Mean liver weight, per cent of body weight	Mean ascorbic acid in liver		
				γ per gram liver	γ per gram body weight	Total γ
4	—	2	4.8	342	16.4	418
5	40	2	4.3	358	15.5	414
3 *	—	3	5.4	315	16.7	348
4	20	3	5.1	355	18.0	392
2	—	5	5.83	290	17.0	477
2	20	5	5.13	359	18.4	507
2	—	6	5.0	301	15.0	381
2	20	6	4.9	370	18.2	444
2	—	8	5.4	304	16.4	413
2	20	8	5.9	338	20.1	516
1	—	9	5.3	329	17.3	401
1	20	9	5.4	390	21.0	472
2	—	12	4.8	232	11.1	256
2	20	12	5.6	280	15.8	464
2	—	15	5.45	263	14.3	452
2	20	15	6.5	358	23.2	592
2	—	19	4.7	305	14.3	364
2	20	19	5.35	365	18.5	463

* The figures from a mouse giving the abnormal amount of 478 γ per gram liver were excluded from these means.

TABLE III: 3,4-BENZPYRENE INTRAPERITONEALLY IN SESAME OIL

Number of mice (CBA ♂)	Dose of 3,4-benzpyrene, mgm.	Interval after injection, days	Mean liver weight, per cent of body weight	Mean ascorbic acid in liver		
				γ per gram liver	γ per gram body weight	Total γ
2	—	2	5.25	329	17.3	348
1	5	2	6.1	383	23.2	511
4	—	3	6.3	320	20.1	438
1	10	3	5.9	372	21.8	438
4	20	3	6.3	423	30.3	449
1	—	5	6.25	342	21.4	492
1	20	5	6.35	404	25.7	380
3	—	6	5.2	278	14.5	326
1	10	6	8.3	396	33.0	661
1	—	9	5.2	321	16.7	375
1	10	9	5.6	293	16.3	413
2	—	13	6.2	242	15.05	396
1	10	13	6.7	219	14.7	374

and those from the mice receiving benzpyrene showed no distinctive pathological changes.

CARCINOGENIC COMPOUNDS OTHER THAN 3,4-BENZPYRENE

All the results given by 8 such compounds, which are summarized in Table IV, and Figs. 3 and 4, were increased in the mice receiving these compounds in every instance except that of 9,10-dimethylanthracene; in the case of 1,2,5,6-dibenzanthracene this rise is not significant on the third, but is distinct on the seventh day.

The carcinogenic compounds 9,10-dimethyl-1,2-benzanthracene, cholanthrene, methylcholanthrene, 1,2,5,6-

dibenzphenanthrene, and stilbestrol all cause increases of from 6 per cent to 135 per cent; of the 11 experiments with these 5 compounds 7 show increases of from 30 to 70 per cent. But one cannot assert any invariable relationship between this effect and the

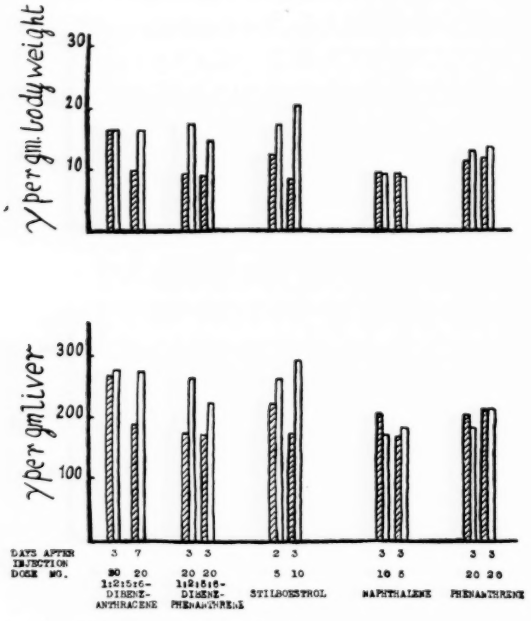

FIG. 3.—MRC male mice. Carcinogenic and noncarcinogenic compounds, intraperitoneally in arachis oil.

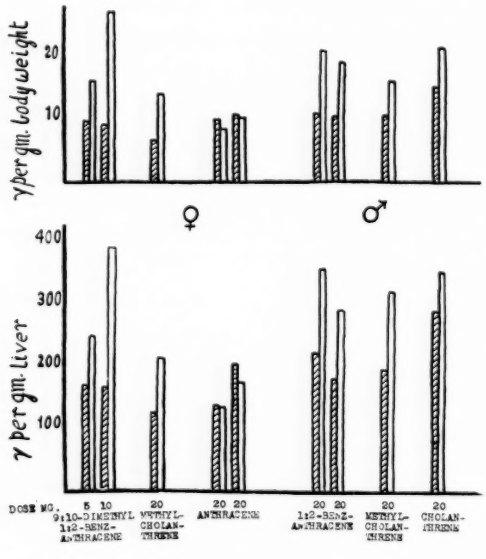

FIG. 4.—CBA male and female mice. Carcinogenic and noncarcinogenic compounds; 3 days after intraperitoneal injection in arachis oil.

carcinogenic power of the compound, for two reasons at least; (a) 9,10-dimethylanthracene, which gave a negative result, is distinctly though not strongly carcinogenic to the skin, while apparently devoid of sarcoma-producing power (7); and (b) 1,2-benzanthracene, which produced an increase of 60 per cent in each of two experiments, is only slightly carcinogenic. This compound induced one epithelioma in 80 mice by application to the skin (3), and its endosuccinate in conjunction with x-radiation gave 3 sarcomas in 10 mice but no tumors in 11 nonirradiated mice (10). The endosuccinate, however, differs from those of many carcinogenic hydrocarbons in being nonhemolytic (11). An experiment now in progress, consisting of the injection of this compound subcutaneously into mice of a strain very susceptible to induced sarcoma (C3H), has so far given negative results. In some other respects 1,2-benzanthracene behaves either in the same way as do carcinogenic compounds, causing deposition of iron-containing material in lymph nodes (6), or in the same way as do the noncarcinogenic compounds, anthracene and phenanthrene, producing effects on the Congo-red index of rabbits (5). Hence the biological properties of this compound appear to overlap those of the carcinogenic and noncarcinogenic hydrocarbons.

Carcinogenic Compounds Acting Chiefly upon the Liver

1. Dimethylaminoazobenzene, injected intraperitoneally in doses of 1 to 5 mgm., in 0.25 cc. oil, was tested (a) by visual titration of extracts of single livers (sesame oil; 3 per cent metaphosphoric acid); and (b) by potentiometric titration of extracts of pooled livers (arachis oil; $M/15$ KH_2PO_4) from 79 mice killed after 2 to 5 days (Table V). The results were not conclusive. The mean concentration of ascorbic acid in the liver in 17 pools was 175 γ per gram in 30 control mice, and 195 γ in 42 receiving the compound, an increase of 11 per cent. Larger doses (10 mgm. and in some cases 5 mgm.) were too toxic.

The experiment in which single livers of mice (CBA males) were extracted showed an increase of 19 per cent:

γ Ascorbic Acid per Gram Liver

Days after injection	Controls	Dimethyl-aminoazo-benzene (5 mgm.)
3	285	369
	261	
6	282	346
9	321	311
	Mean 287 = 100	342 = 119

2. Experiments with 2,2′-azonaphthalene are described under "Saturation Tests" below.

Noncarcinogenic Hydrocarbons

Three compounds of this class, naphthalene, anthracene, and phenanthrene, were tested by potentiometric

TABLE IV: CARCINOGENIC COMPOUNDS OTHER THAN 3,4-BENZPYRENE

Compound, and intraperitoneal dose in mgm.		Strain and sex of mice, and number pooled	Interval after injection, days	Mean liver weight, per cent of body weight	Mean ascorbic acid in liver: γ per gram liver	γ per gram body weight	Total γ
Controls		CBA ♀ 5	3	5.8	170	9.9	167
9,10-Dimethyl-1,2-benzanthracene	5	" " 5	3	6.5	250	16.3	330
Controls		" " 5	3	5.4	167	9.0	166
9,10-Dimethyl-1,2-benzanthracene	10	" " 6	3	7.0	392	27.5	459
Controls		" " 6	3	5.4	125	6.7	135
Methylcholanthrene	20	" " 6	3	6.6	213	14.1	272
Controls		" ♂ 4	3	5.5	198	10.9	230
Methylcholanthrene	20	" " 3	3	5.2	323	16.6	404
Controls		MRC ♀ 4	3	7.9	158	12.5	313
Cholanthrene	20	" " 4	3	6.7	261	17.4	472
Controls		" " 4	3	4.7	227	10.7	243
Cholanthrene	20	" " 4	3	6.4	240.5	15.3	353
Controls		CBA ♂ 4	3	5.2	293	15.4	396
Cholanthrene	20	" " 4	3	6.1	358	22.0	501
Controls		MRC ♂ 5	3	6.2	270	16.6	356
1,2,5,6-Dibenzanthracene	20	" " 5	3	6.0	278	16.6	381
Controls		" " 5	7	5.3	188	10.0	239
1,2,5,6-Dibenzanthracene	20	" " 5	7	5.9	277	16.4	368
Controls		" " 4	3	5.3	176	9.4	244
1,2,5,6-Dibenzphenanthrene	20	" " 5	3	6.7	262	17.7	484
Controls		" " 5	3	5.3	172	9.1	247
1,2,5,6-Dibenzphenanthrene	20	" " 5	3	6.6	226	15.0	382
Controls		" ♀ 5	3	6.4	214	13.8	353
9,10-Dimethylanthracene	20	" " 5	3	7.2	212	15.3	428
Controls		" " 4	7	6.4	266	17.1	359
9,10-Dimethylanthracene	20	" " 4	7	5.5	244	13.6	310
Controls		CBA ♂ 4	3	5.9	183	10.8	234
1,2-Benzanthracene	20	" " 4	3	6.7	295	19.8	375
Controls		" " 5	3	5.0	224	11.1	282
1,2-Benzanthracene	20	" " 5	3	5.9	360	21.5	504
Controls		MRC ♂ 5	2	5.5	224	12.4	343
Stilbestrol	5	" " 5	2	6.7	262	17.5	576
Controls		" " 5	3	5.0	175	8.8	224
Stilbestrol	10	" " 4	3	7.0	292	20.4	531

TABLE V: DIMETHYLAMINOAZOBENZENE

Strain and sex of mice, and number pooled	Dose, mgm.	Interval after injection, days	Mean liver weight, per cent of body weight	Mean ascorbic acid in liver: γ per gram liver	γ per gram body weight	Total γ
CBA ♀ 6	Controls	2	5.4	165	8.9	179
" " 6	3	2	5.6	198	11.2	232
" " 3	Controls	2	6.1	222	13.5	244
" " 3	3	2	7.1	183	12.9	181
" " 3	4	2	7.1	236	16.8	307
" " 3	2	2	5.6	196	11.0	221
" " 3	3	2	5.8	220	12.6	224
" " 6	Controls	3	7.2	134	9.7	182
" " 3	2	3	8.8	186	16.4	324
" " 3	3	3	6.7	177	11.9	281
" " 3	Controls	5	5.7	218	12.4	211
" " 3	3	5	7.4	157	11.7	218
" " 3	4	5	6.0	211	12.7	257
" ♂ 6	Controls	2	4.9	156	7.7	139
" " 6	1	2	5.1	176	8.9	158
" " 6	2	2	5.4	208	11.3	229
" " 6	Controls	3	6.0	202	12.2	265

titration of extracts in $M/15$ KH_2PO_4 of the pooled livers of from 4 to 6 mice, 3 days after intraperitoneal injection in 0.25 cc. arachis oil. In no case was there any significant increase in the concentration of ascorbic acid in the liver; the figures for this quantity are actually lower in the treated than in the control mice in 5 of the 6 experiments (Table VI). Napthalene is the most toxic of these compounds; 10 mgm. of it made the mice shaky and limp, but 5 mgm. had no visible effect.

An experiment in which single livers of mice (CBA males) receiving anthracene (20 mgm. in 0.2 cc. sesame oil, intraperitoneally) were extracted with 3 per cent metaphosphoric acid gave a confirmatory result:

γ Ascorbic Acid per Gram Liver

Days after injection	Controls	Anthracene
1	316	340
	253	254
4	320	232
		256
	Mean 296	271

Protocols 1 and 2 show that marrow juice can destroy completely the substance in the livers both of normal mice and of those receiving 3,4-benzpyrene, which reacts with 2,6-dichlorophenolindophenol, and this destruction is from 10 to 20 times as rapid (see protocol 2) as that which occurs spontaneously. There seems to be no doubt, therefore, that the substance in question is ascorbic acid.

In similar experiments the reducing substance in the liver extract ($M/15$ KH_2PO_4) after administration of anthracene (20 mgm. in 0.25 cc. arachis oil intraperitoneally 3 days before) was lowered by marrow juice to one-tenth of the initial value in one experiment, and to zero in the other, in 30 minutes at 26°; and in one of several experiments with dimethylaminoazobenzene (1.0 and 2.0 mgm. intraperitoneally) complete removal in 30 minutes was brought about.

Glutathione

In the analyses of single livers of mice glutathione was estimated by titration with iodine after the end-point with 2,6-dichlorophenolindophenol was reached, with the following results:

Table VI: Noncarcinogenic Hydrocarbons

Compound, and intraperitoneal dose in mgm.	Strain and sex of mice, and number pooled	Mean liver weight, per cent of body weight	Mean ascorbic acid in liver: γ per gram liver	γ per gram body weight	Total γ
Controls	MRC ♂ 5	4.8	206	9.8	266
Naphthalene 10	" " 4	5.5	171	9.4	198
Controls	" " 5	5.7	170	9.7	238
Naphthalene 5	" " 5	4.9	183	9.0	257
Controls	" " 5	5.7	206	11.7	268
Phenanthrene 20	" " 4	7.2	184	13.3	309
Controls	" " 5	5.6	217	12.1	271
Phenanthrene 20	" " 5	6.5	216	14.0	324
Controls	CBA ♀ 6	7.2	140	10.1	200
Anthracene 20	" " 6	6.1	138	8.5	157
Controls	" " 5	5.3	206	10.9	236
Anthracene 20	" " 6	5.8	177	10.3	209

Nature of Reducing Substance

The question had to be considered whether the additional amount of substance reacting with 2,6-dichlorophenolindophenol that is found in extracts of the livers of mice receiving hydrocarbons is actually ascorbic acid. This was tested (a) by checking the visual titration by titration under potentiometric control (4); and (b) by adding ascorbic acid oxidase, present in the juice obtained by thawing vegetable marrow after freezing for 2 days (9), which would remove the reducing agent if this consisted wholly of ascorbic acid. All preparations of marrow juice are not equally effectual, but some samples gave results such as that shown in the following protocols (Table VII).

Table VIII: Glutathione in Liver

CBA ♂	Mean cc. 0.01 N iodine per gram liver
20 controls, no injection	1.00
Sesame oil (0.1 to 0.25 cc.) used in all injections	
18 oil subcutaneously	0.88
18 oil intraperitoneally	0.93
31 3,4-benzpyrene, subcutaneously and intraperitoneally 5 to 40 mgm.	0.985
3 dimethylaminobenzene, intraperitoneally 5 mgm.	0.946
4 anthracene, intraperitoneally 20 mgm.	1.086

TABLE VII: ASCORBIC ACID OXIDASE

Protocol 1

Sept. 11, 1942 5 ♂ CBA received intraperitoneally 0.25 cc. arachis oil each (A)
4 ♂ " " " " " " + 20 mgm. 3,4-benzpyrene each (B)
Sept. 14, 1942 Killed. Mean weight of liver: A, 1.18 gm.: B, 1.30 gm.

Livers ground in 20 cc. $M/15$ KH_2PO_4, spun; residue twice stirred with 15 cc. buffer and spun; combined supernatants made up to 52.0 cc. (A) and 53.5 cc. (B)

	cc. Dye (potentiometric titration) A	B
(a) 10 cc. extract + 1 cc. 50% metaphosphoric acid, spun; supernatant + 10 cc. buffer titrated	0.485	0.655
(b) 10 cc. extract + 10 cc. buffer } 15′ at 26°	0.50	0.685
(c) " " " " marrow juice } 15′ at 26°	0.00	0.00
(d) " " " " buffer } 35′ at 26°	0.450	0.655
(e) " " " " marrow juice } 35′ at 26°	0.00	0.00

(b), (c), (d), (e) were placed in open Erlenmeyer flasks and were spun + 2 cc. 50% metaphosphoric acid before titration.

	Mean ascorbic acid γ per liver	γ per gram liver
A mice (Controls)	202	171
B mice (3,4-Benzpyrene)	350	270

Protocol 2

Sept. 15, 1942 10 ♂ CBA Controls (A) } injection
10 ♂ " 3,4-Benzpyrene (B) } as above
Sept. 18, 1942 Killed. Mean weight of liver: A, 1.27 gm.; B, 1.425 gm.

Livers ground in 50 cc. $M/15$ KH_2PO_4; spun + 25, 25, and 10 cc. made up to 110 cc. (A) and 112 cc. (B)

(a) 10 cc. extract	0.65	0.83
(b) " " " + 70 cc. buffer } 19′ at 26°	0.54, 0.55	0.81, 0.81
(c) " " " " " marrow juice } 19′ at 26°	0.06, 0.055	0.06, 0.035
(d) " " " " " buffer } 47′ at 26°	0.49, 0.50	0.76, 0.76
(e) " " " " " marrow juice } 47′ at 26°	0.00, 0.00	0.03, 0.02

(a), (b), (c), (d), (e) treated as above.
Blank. 35 cc. marrow juice + 3.5 cc. 50% metaphosphoric acid, spun. 0.045
Mean of 3 values

	Mean ascorbic acid γ per liver	γ per gram liver
A mice (Controls)	286	225
B mice (3,4-Benzpyrene)	372	261

Thus, under the conditions of these experiments, the concentration of glutathione in the liver is not affected distinctly by the compounds used, even in doses, in most cases, of 20 mgm.

RATIO OF LIVER WEIGHT TO BODY WEIGHT

During the investigations described below we had at first assumed that the injections of sesame or arachis oil had no effects that were significant from the present point of view. But examination of the figures in a series (CBA males) where comparison was possible gave the following mean results (8):

	Liver weight, per cent of body weight	Ascorbic acid γ per gram liver	Ascorbic acid γ per gram body weight
Uninjected	4.8	349	16.7
Injected (Sesame oil subcutaneously or intraperitoneally)	5.40	306	16.4

Hence sesame oil (a) decreases the concentration of ascorbic acid in the liver, and (b) increases the ratio of liver weight to body weight; this latter change might of course be due either to a rise in liver weight or to a fall in body weight. An experiment designed to test this point, in which CBA male mice were weighed daily after subcutaneous or intraperitoneal injections of sesame oil, showed no alterations in body weight different from those shown by controls.

The data obtained in these experiments upon the ratio of liver weight to body weight are summarized in Table IX.

The results in Table IX show, in the most complete series, namely the CBA males, (a) that the injection of sesame or arachis oil alone raises the ratio; (b) that intraperitoneal injection is much more effectual than subcutaneous; and (c) that carcinogenic hydrocarbons cause a further increase beyond that due to the oil.

The table shows further (d) that other mice are affected in the same way by carcinogenic hydrocarbons; (e) that the noncarcinogenic hydrocarbons, and dimethylaminoazobenzene, give irregular results; and (f) that these effects are less distinct or absent in female mice, in which normally the ratio is higher than in the male (8).

This enlargement of the liver tends to counteract the increase in the amounts of ascorbic acid per gram liver, produced by carcinogenic compounds. Hence this increase in ascorbic acid is sometimes shown more distinctly by the amounts per gram body weight (Tables II, III, and IV, and Figs. 1, 2, and 3).

TABLE IX: RATIO OF LIVER WEIGHT TO BODY WEIGHT

			Liver weight, per cent of body weight
CBA ♂	20	Uninjected	4.80
	21	Controls. Sesame oil subcutaneously	5.09
	22	" " " + benzpyrene	5.18
	13	Controls. " " intraperitoneally	5.79
	10	" " " " "	6.41
	17	Controls. Arachis oil intraperitoneally	5.35
	16	Methylcholanthrene, cholanthrene, 1,2-benzanthracene, in arachis oil intraperitoneally	5.99
	12	Controls. Arachis oil intraperitoneally	5.51
	23	Dimethylaminoazobenzene " " " "	5.37
CBA ♀	35	Uninjected	5.76
	16	Controls. Arachis oil intraperitoneally	5.70
	17	9,10-Dimethyl-1,2-benzanthracene, methylcholanthrene, in arachis oil intraperitoneally	6.73
	11	Controls " " "	6.39
	12	Anthracene " " " "	5.99
	18	Controls " " "	6.18
	30	Dimethylaminoazobenzene " " " "	6.56
MRC ♂		Uninjected, none	
	29	Controls Arachis oil intraperitoneally	5.44
	29	1,2,5,6-Dibenzanthracene in " " "	6.51
		1,2,5,6-Dibenzphenanthrene " " " "	
		Stilbestrol " " " "	
	20	Controls " " "	5.42
	18	Naphthalene, phenanthrene, " " " "	5.95
MRC ♀			
Buff	9	Uninjected	6.38
	12	Arachis oil or saline intraperitoneally	6.13
White	17	Controls Arachis oil intraperitoneally	6.42
	17	9,10-Dimethylanthracene, cholanthrene, in " " "	6.57

SATURATION TESTS

2,2′-Azonaphthalene, which produces cholangioma and hepatoma and is not known to produce any other tumors (2), was given (10 mgm. intraperitoneally) to male stock mice obtained from a breeder, and after 3 days extracts (3 per cent metaphosphoric acid) of the pooled livers of 4 mice were titrated by the potentiometer (Table X). The first experiment showed an increase of 42 per cent (394 as against 278 γ per gram): a repetition gave the very high figure of 376 γ per gram liver in the control mice, a concentration higher than that in the mice receiving the compound (331 γ). The comparison of these two results suggested that the increases, usually of 30 to 70 per cent, produced by carcinogenic compounds in mice of other strains (Tables II, III, IV, and Figs. 1-4) could occur only if there were some deficiency in the store of ascorbic acid already present. To test this possibility the strongly carcinogenic compound 9,10-dimethyl-1,2-benzanthracene, which caused a considerable increase (Table IV and Fig. 4) in CBA female mice whose normal level of ascorbic acid in the liver is low (8), was given to these same stock mice (10 mgm. intraperitoneally; 4 mice pooled 3 days later) and no increase occurred. The result of this comparison (Table X) supports the suggestion made above, that the increase of ascorbic

acid brought about by carcinogens is analogous to the filling-up of a partially filled vessel. To test this question, some preliminary saturation tests were made, as follows:

Series A.—Four stock males injected subcutaneously with 1.0 mgm. ascorbic acid in 0.25 cc. water on 3 consecutive days and given water to drink containing 4 mgm./cc. ascorbic acid; killed on third day and treated as in preceding experiments. There was practically no increase in the ascorbic acid (Table X).

Series B.—Same as series A, but 5 mgm. ascorbic acid in 0.25 cc. saline injected subcutaneously on 2 consecutive days; saline containing 4 mgm./cc. ascorbic acid given to drink; killed on second day. The result confirms that of series A.

Note on the Potentiometric Method

Harris, Mapson, and Wang (4) have described a method for the electrometric determination of ascorbic acid, based on the use of a mercury-coated platinum electrode, that gives excellent results with solutions of pure ascorbic acid, plant extracts, and, in our experience, human and animal urines. Preliminary experiments showed that it was unsuitable for use with extracts of mouse liver because, owing to the large amounts of interfering substances present in such extracts, the electrode does not reach equilibrium rapidly. In all the present experiments, therefore, we employed the alternative technic described by these authors in which a bright platinum electrode is used.

Table X: Saturation Tests

	Mice	Mean ascorbic acid in liver: γ per gram liver	γ per gram body weight	Total, γ
2,2'-Azonaphthalene				
Controls	4 Stock ♂	278	15.7	388
Injected, 10 mgm.	" "	394	22.9	629
Controls	" "	376	23.4	592
Injected, 10 mgm.	" "	331	20.2	515
9,10-Dimethyl-1,2-benzanthracene				
Controls	5 CBA ♀	170	9.9	167
Injected, 5 mgm.	" "	250	16.3	330
Controls	" "	167	9.0	166
Injected, 10 mgm.	6 "	392	27.5	459
Controls	4 Stock ♂	381	27.5	742
Injected, 10 mgm.	" "	379	24.8	608
Saturation experiment				
Series A.				
Controls	" "	373	23.4	568
Ascorbic acid	" "	386	22.4	533
Series B.				
Controls	" "	323	21.5	445
Ascorbic acid	" "	344	20.8	453

The similarity of the maximum concentrations (392, 381, 379, 394, 376, 373, 386 γ) suggests that 350 to 400 γ per gram liver is a limiting concentration of ascorbic acid in these mice. Values above these are seldom observed, presumably because excretion of ascorbic acid into the urine rapidly restores the normal level in the liver.

The degree of saturation of the liver tissue with ascorbic acid varies apparently from strain to strain. We made no further experiments with these stock mice, and do not know under what conditions they may have lived elsewhere. Other mice from the same source have shown much lower amounts of ascorbic acid (8, Fig. 4). We have not ascertained the maximum concentration of liver ascorbic acid that can be achieved in other strains (*e.g.*, CBA, C3H, dba, RIII), but the choice of strain is clearly of importance in experiments designed to show increases in the normal concentration. This question is under further investigation here.

The endpoint in all cases was quite sharp and marked by a rise in potential of about 100 mv.

DISCUSSION

1. Several lines of investigation suggest a possible relation between ascorbic acid and carcinogenic factors, and also a contrast between ascorbic acid and glutathione in this respect. Such evidence consists of:

(a) The aerobic oxidation of carcinogenic (and other) hydrocarbons in the presence of ascorbic acid (12).

(b) The higher concentration of ascorbic acid in the liver of mice of some high-cancer strains at any rate, and the absence of any such difference between high-cancer and low-cancer strains in regard to glutathione (8).

(c) The effect of 3,4,5,6-dibenzcarbazole (1) which, in doses (0.25 and 0.5 mgm.) causing a high mortality and in some cases great enlargement and considerable histological change of the liver, produced

increases of glutathione up to twice the normal amount, but no definite effect upon the ascorbic acid.

(d) In contrast to these results those recorded in this paper show that many carcinogenic hydrocarbons in doses of 20 mgm. produce, without mortality or obvious changes in the liver, increases up to 70 per cent in the concentration of ascorbic acid, while the compound of this class tested upon this point, namely 3,4-benzpyrene, did not affect the glutathione content of the liver.

2. The results presented in this paper show a contrast between the carcinogenic and noncarcinogenic compounds tested. The former (3,4-benzpyrene, 1,2,5,6-dibenzanthracene, 1,2,5,6-dibenzphenanthrene, 9,10-dimethyl-1,2-benzanthracene, cholanthrene, methylcholanthrene) cause an increase in the concentration and total amount of ascorbic acid in the liver, while the 3 noncarcinogenic compounds (naphthalene, anthracene, phenanthrene) have not this effect. We cannot, of course, say whether this contrast will hold good for other compounds of the two classes, but experiments upon this question are being continued.

SUMMARY

1. 3,4-Benzpyrene injected in sesame or arachis oil subcutaneously or intraperitoneally, and other carcinogenic compounds (9,10-dimethyl-1,2-benzanthracene, 1,2,5,6-dibenzanthracene, 1,2,5,6-dibenzphenanthrene, cholanthrene, methylcholanthrene) injected in arachis oil intraperitoneally, caused an increase in the concentration of ascorbic acid in the liver of mice. Dimethylaminoazobenzene produced a less definite increase, and the three noncarcinogenic compounds tested (naphthalene, anthracene, phenanthrene) caused no increase at all. 1,2-Benzanthracene had an effect similar to that of the strongly carcinogenic compounds named above, while 9,10-dimethylanthracene, which is carcinogenic to the skin, gave a negative result. The identity with ascorbic acid of the reducing substance estimated in these experiments was shown by the use of ascorbic acid oxidase from vegetable marrow.

2. The glutathione content of the liver was not affected distinctly by the compounds (3,4-benzpyrene, dimethylaminoazobenzene, anthracene) tested in this respect.

3. In male mice injection of sesame oil subcutaneously or intraperitoneally, and of arachis oil intraperitoneally, caused an increase in the weight of the liver, and the addition of carcinogenic hydrocarbons to the oil caused a further increase.

4. Some evidence was obtained that these increases in ascorbic acid in the liver occur only if there is some deficiency in the store of the compound already present.

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On the Role of Thymus, Spleen, and Gonads in the Development of Leukemia in a High-Leukemia Stock of Mice*

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Little knowledge exists concerning the factors that lead to the development of spontaneous leukemia in a high-leukemia stock of mice. It is known that the occurrence of the disease is governed by factors of heredity (2), that the leukemic cells are not present as such in the preleukemic period (13), and that the malignant transformation, which takes place at about the fifth to eighth month of life, is postponed or prevented by underfeeding (13). Furthermore, genetic studies (4) have suggested that certain cells with high neoplastic potentialities may be present in a dormant form in mice with hereditary susceptibility to leukemia.

It has been noted that the thymus is almost always involved in leukemia affecting the high leukemia stock (Ak) under investigation. It is sometimes the sole organ involved, while the degree of leukemic infiltration in spleen and lymph nodes is variable and the bone marrow often appears normal. Thus the thymus may be the most common site of potentially neoplastic lymphocytes, and consequently it seemed advisable to ascertain what effect its removal at an early age would have on the incidence of leukemia. The spleen is another lymphoid organ, and if neoplasms originate in it from preformed cells destined to become malignant at a later age, then removal of the spleen during the preleukemic period might lower the incidence of this disease. It is also possible that these organs influence the occurrence of leukemia in some other way and that this would be revealed by their removal.

The relation of the sex organs to leukemia in mice is indicated by the greater frequency of this disease in female animals (2) and by the experimental production of leukemia by sex hormones. As early as 1937 Gardner (5) and Lacassagne (7) had noted the frequent occurrence of leukemia in estrogen-treated mice of stocks in which leukemia was uncommon. This lead was followed up by Gardner and his associates (6); Shimkin, Grady, and Andervont (15); and Bischoff, Long, Rupp, and Clarke (1), all of whom have shown under well-controlled experimental conditions that the incidence of leukemia can be greatly enhanced in most stocks of mice by prolonged administration of relatively large doses of natural or synthetic estrogenic hormones. Marine and Rosen (8) found that lymphomatosis was more common in castrated than in normal male fowls.

In order to learn more about the part played by sex hormones on the incidence of lymphoid neoplasms in the high leukemia stock Ak, both males and females of this stock were subjected to gonadectomy and observed until natural death.

MATERIAL AND METHODS

All experiments were performed on mice of the high-leukemia stock Ak, inbred in this laboratory during the past 15 years. There is from time to time a slight variability in the percentage incidence of leukemia for different sublines of this stock, and therefore each group of experimental animals was matched with related mice of the same generation and subline as controls. The incidence of spontaneous leukemia in the various sublines carried is approximately the same.

All mice were kept under as nearly identical conditions as possible until natural death. All animals were autopsied; when the gross diagnosis was doubtful, microscopic sections were made from liver, spleen, lymph nodes, bone marrow, and sometimes thymus.

The thymectomies were performed on mice 31 to 71 days of age because the operative mortality was very high among younger mice. The technic used was essentially the same as that described by Segaloff for the rat (14). At autopsy the mediastinum was carefully explored.

The splenectomies were performed on mice 28 to 48 days of age. A lateral incision in the region of the spleen was made through the skin and ab-

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dominal wall. The spleen was drawn through this opening, the vessels ligated, and the organ excised.

The ovariectomies were performed on animals 23 to 56 days old. The ovaries were exposed by a lumbar incision and removed, together with the oviducts and a portion of the fat body. The orchidectomies were performed on mice 20 to 56 days old. A scrotal incision was made, the vas deferens and testicular vessels were ligated with a single ligature, and the testis and epididymis removed.

Animals that died before the appearance of the first case of leukemia were discounted from the experiment. Accordingly, in the thymectomy and gonadectomy groups and their controls are included animals living 6 months or longer; while in the splenectomy group and its controls are included animals living 5 months or longer. The loss in each of the female groups was small (1 to 3 mice), while among the male animals it was relatively high (3 to 27 mice) because of fighting at 3 to 5 months of age.

Experimental Results and Their Discussion

The effect of thymectomy is seen in Figs. 1 and 2, that of splenectomy in Figs. 3 and 4, that of ovariectomy in Fig. 5, and that of orchidectomy in Fig. 6. The results of all experiments are summarized in Table I.

Table I: Incidence of Leukemia in Experimental and Control Mice

Mice	Thymectomy				Splenectomy				Gonadectomy			
	Females		Males		Females		Males		Females		Males	
	Experimental	Controls	Experimental	Controls	Experimental	Controls	Experimental	Controls	Experimental	Controls	Experimental	Controls
No. in experiment	40	44	46	44	71	75	87	94	56	67	83	67
No. with leukemia	3	34	5	27	38	43	40	48	25	50	50	35
Percentage with leukemia	*8*	*77*	*11*	*61*	*54*	*57*	*46*	*51*	*45*	*74*	*60*	*52*

The reduction in the incidence of leukemia as the consequence of *thymectomy* is conspicuous in both male and female mice. Never have such low percentage figures for leukemia (11 per cent and 8 per cent) been observed in normal mice of this stock. Similar low figures (10.1 per cent) have been encountered in only one experimental series, that is, in mice that were underfed (13), and the question arises whether the effects of thymectomy are due to malnutrition. In the underfeeding experiments (13) caloric reduction of an adequate diet began at the age of 4 weeks, and the animals were definitely underweight and small in size as compared with the controls, whereas the animals of the present series were almost as large as the controls, though they appeared to be slightly underweight. The animals of this experimental series were not weighed. Precise data on the relation of thymus to body weight will be forthcoming from experiments now in progress. The underfed animals remained sterile (13), whereas the thymectomized animals bred well. In both underfed and thymectomized animals mortality during the first few months of life was greater than in the controls. Among the underfed mice the excessive mortality occurred at 1 to 6 months of age, and among the thymectomized mice at 6 to 10 months. Figs. 1 and 2 indicate that a larger proportion of thymectomized animals reached an old age than among the controls, and this is also true for the underfed animals (13).

Retardation of development of rats by underfeeding causes involution of the thymus and an increase in the ratio of body weight to thymus weight; after about 250 days, however, the difference in these ratios is slight and the figures are even higher in normally fed than in retarded animals (12). It is thus conceivable that the decrease in the incidence of lymphoid neoplasms resulting from underfeeding is indirect and is the immediate consequence of involution of the thymus. However, neoplasms other than lymphomatosis are likewise infrequent among the underfed animals (11), and if the effects of underfeeding are related to atrophy of the thymus it must be supposed that this organ contains substances that influence neoplastic tendencies in general. Thus there are at least three possibilities to explain the results of thymectomy: (a) removal of sites of origin of leukemia, (b) developmental disturbance not specifically due to absence of thymus, (c) lack of hypothetical thymic hormones. The last assumption seems unlikely, since the administration of estrogen brings about an increased incidence of leukemia with involution of the thymus (6). An interpretation of the consequences of thymectomy, here described, must therefore be postponed until data are forthcoming from experiments such as that on the effect of thymic extract in normal and thymectomized animals.

Splenectomy had no significant effect on the incidence of leukemia or on the longevity of the animals (Figs. 3 and 4). The incidence of leukemia was slightly lower among splenectomized animals than among the controls, but there was a slightly higher incidence of death from causes other than leukemia among the splenectomized animals 5 to 9 months

of age. The findings suggest than in the majority of cases the involvement of the spleen in leukemia is a secondary process. This conclusion is in harmony with the results of experiments aimed to determine the onset of leukemia by bioassays of various organs (13).

The spleen is generally not regarded as an organ essential for life, and we are not familiar with any data to indicate that the life span of animals and

on mammary gland carcinoma Cori (3), following up the observations of Leo Loeb, found that the incidence of the disease was reduced from 78.5 per cent to 10 per cent in animals ovariectomized at 2 to 6 months of age and to zero per cent in those ovariectomized at 15 to 22 days. In the experiments of Murray (9) the incidence of mammary tumors among female mice spayed at 4 to 6 weeks was 17.1 per cent, as compared with 11.5 per cent in normal non-

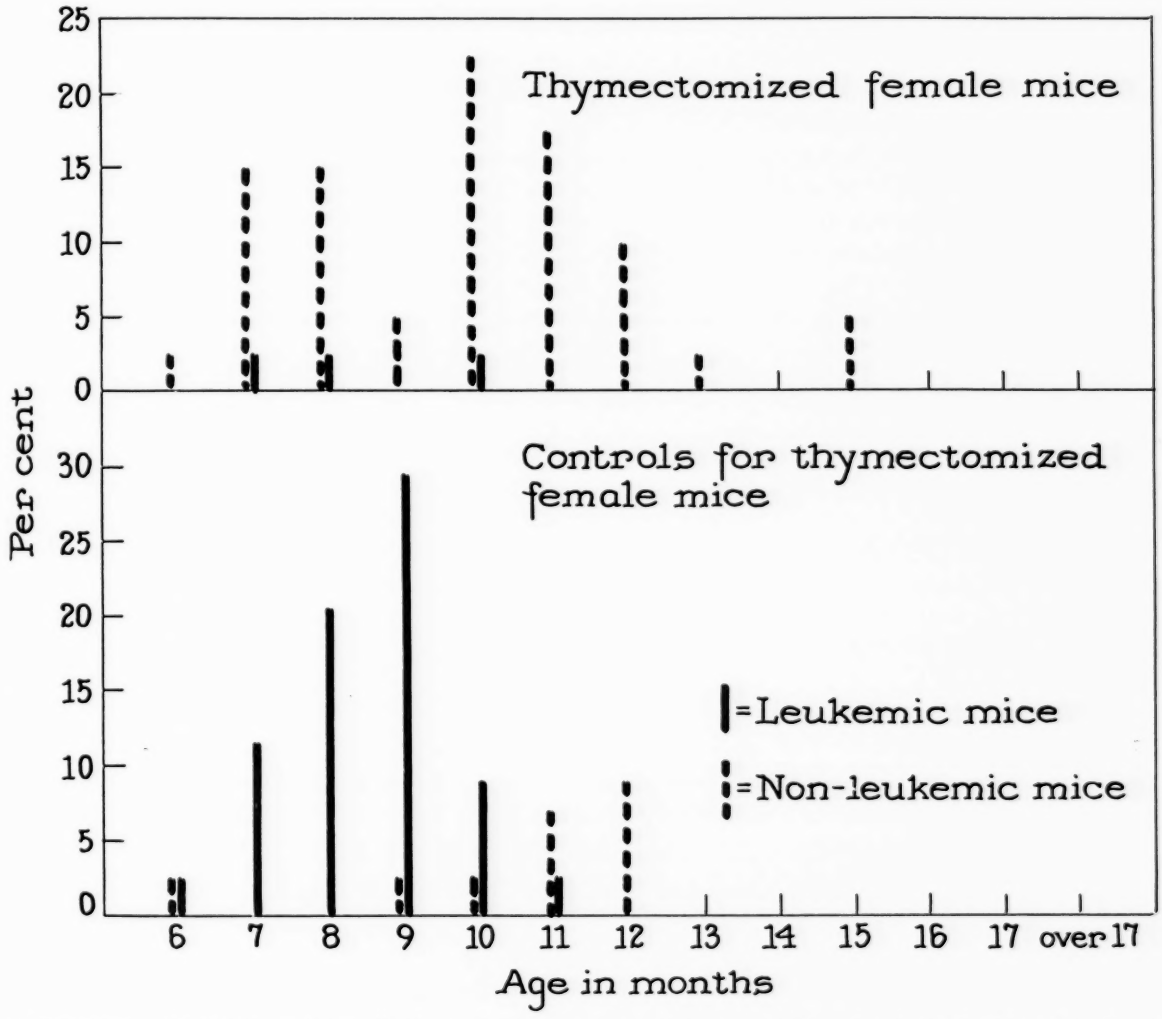

FIG. 1.—Incidence of leukemia and mortality from other causes among thymectomized female mice of the Ak stock and controls.

the incidence of disease are notably altered when splenectomy is performed in immature adult animals that are not carriers of latent infections. The present experiments failed to reveal any such effect resulting from splenectomy.

Fig. 5 shows that the incidence of spontaneous leukemia was reduced in mice by *ovariectomy,* and Fig. 6, that it was raised by *orchidectomy*. Gonadectomy was performed at 20 to 56 days, and it is probable that the animals had by this time been stimulated by some androgenic or estrogenic hormones. In studies

breeding females and 80 per cent in breeding females. The inference can be drawn from these studies that some hormones instrumental in the production of mammary cancer have already been secreted at 4 to 6 weeks of age, or slightly earlier, in sufficient quantities to influence the appearance of this neoplasm in highly susceptible animals.

The magnitude of the changes resulting from gonadectomy was not great in our experiments, but appeared definite. The incidence of leukemia in spayed females was 45 per cent as compared with 74 per

cent in the controls, while the reverse effect was noted among orchidectomized animals, the incidence of leukemia rising to 60 per cent in the experimental males as compared with 52 per cent in the controls. The results of orchidectomy alone are probably not statistically significant, but it is the only instance thus far known to us in which leukemia was higher in a group of mice that had been subjected to an operative procedure than among the corresponding controls. Moreover, the finding is in harmony with the observations (a) that the incidence of leukemia is definitely higher among female than among male mice, and (b) that ovariectomy lowers considerably the incidence of leukemia.

Pybus and Miller (10) noted that castration delayed the onset of leukemia in one of their strains. In the experiments of Gardner and his associates (6) intact or breeding animals had no more lymphomas than did castrated or virgin females. However, the incidence of lymphoid tumors was not higher in female than in male mice of the stocks studied by them, indicating that in these stocks the role of sex hormones in the spontaneous occurrence of this disease is negligible. The tendency for estrogens to be more effective in increasing the incidence of lymphoid tumors in some strains than in others had been noted by Gardner, Dougherty, and Williams (6), and this could not be correlated with a concomitant tendency to acquire mammary, hypophyseal, or testicular tumors in response to estrogens. The lymphoma-producing action was inhibited by testosterone propionate (6).

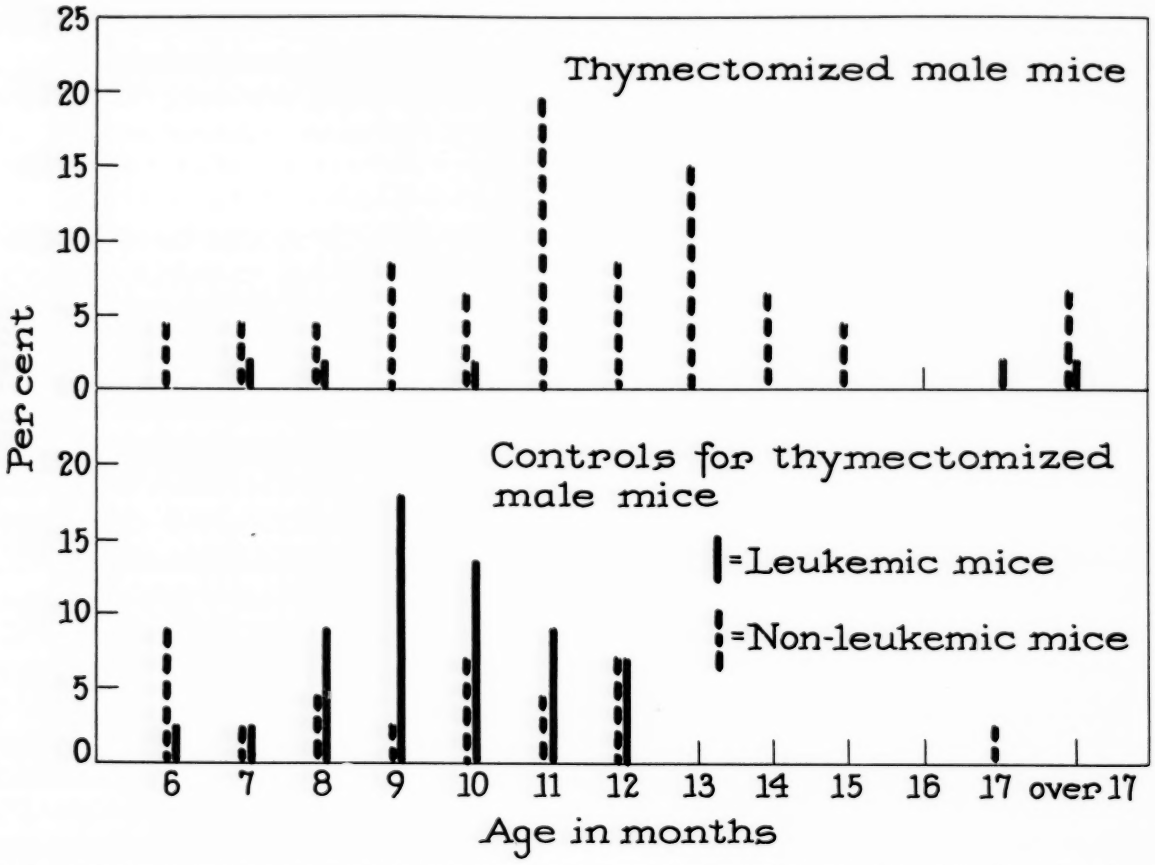

FIG. 2.—Incidence of leukemia and mortality from other causes among thymectomized male mice of the Ak stock and controls.

SUMMARY

Removal of the thymus from mice of a high-leukemia stock (Ak) at 31 to 71 days of age resulted in a reduction of the incidence of spontaneous leukemia from 77 to 8 per cent in females, and from 61 to 11 per cent in males.

Leukemia is more common in female than in male mice. The incidence of this disease was lowered from 74 to 45 per cent by ovariectomy at 23 to 56 days. Among males subjected to orchidectomy at 20 to 56 days the incidence of leukemia was 60 per cent, as compared with 52 per cent among the controls of this experimental series.

Splenectomy at 28 to 48 days did not significantly alter the incidence of spontaneous leukemia.

The role of thymus, spleen, and gonads in the causation or evolution of spontaneous leukemia is discussed in the light of the data here presented.

We gratefully acknowledge the assistance of Dr. J. A. Saxton, Jr. and Miss Alice Klauber in the necessary operations.

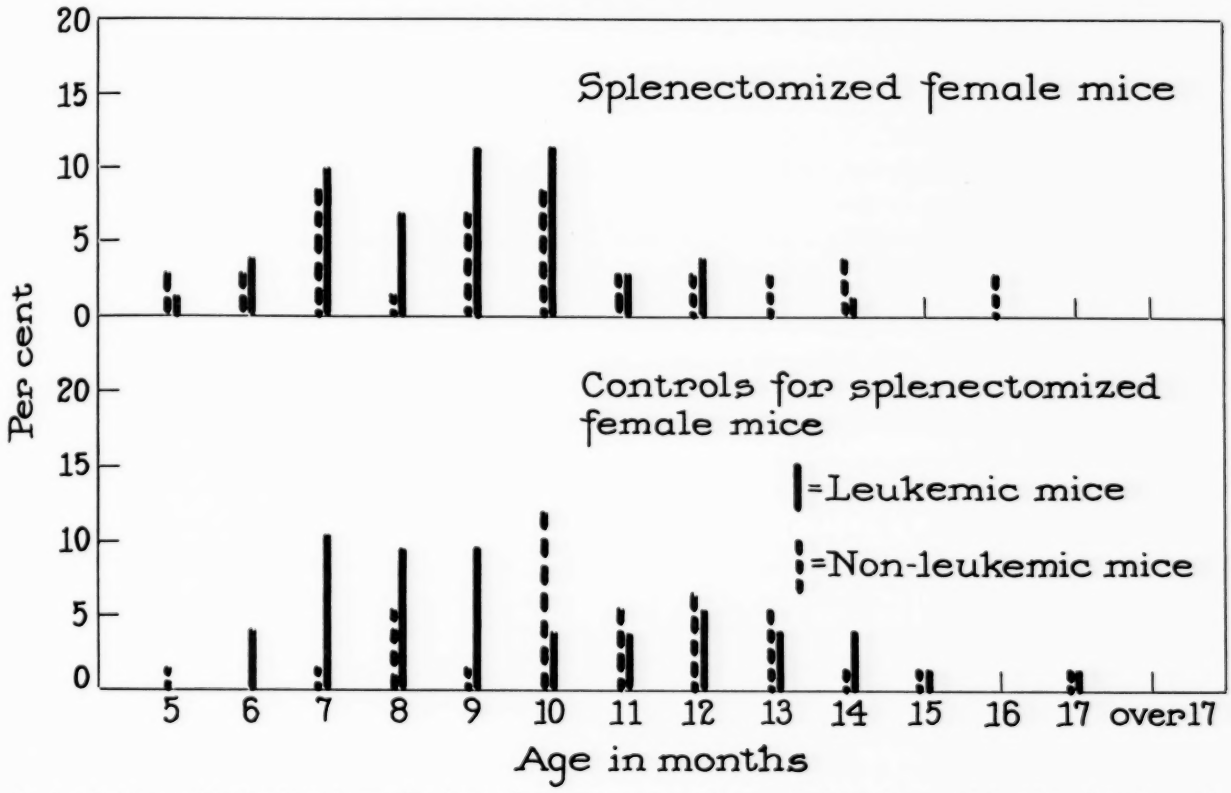

FIG. 3.—Incidence of leukemia and mortality from other causes among splenectomized female mice of the Ak stock and controls.

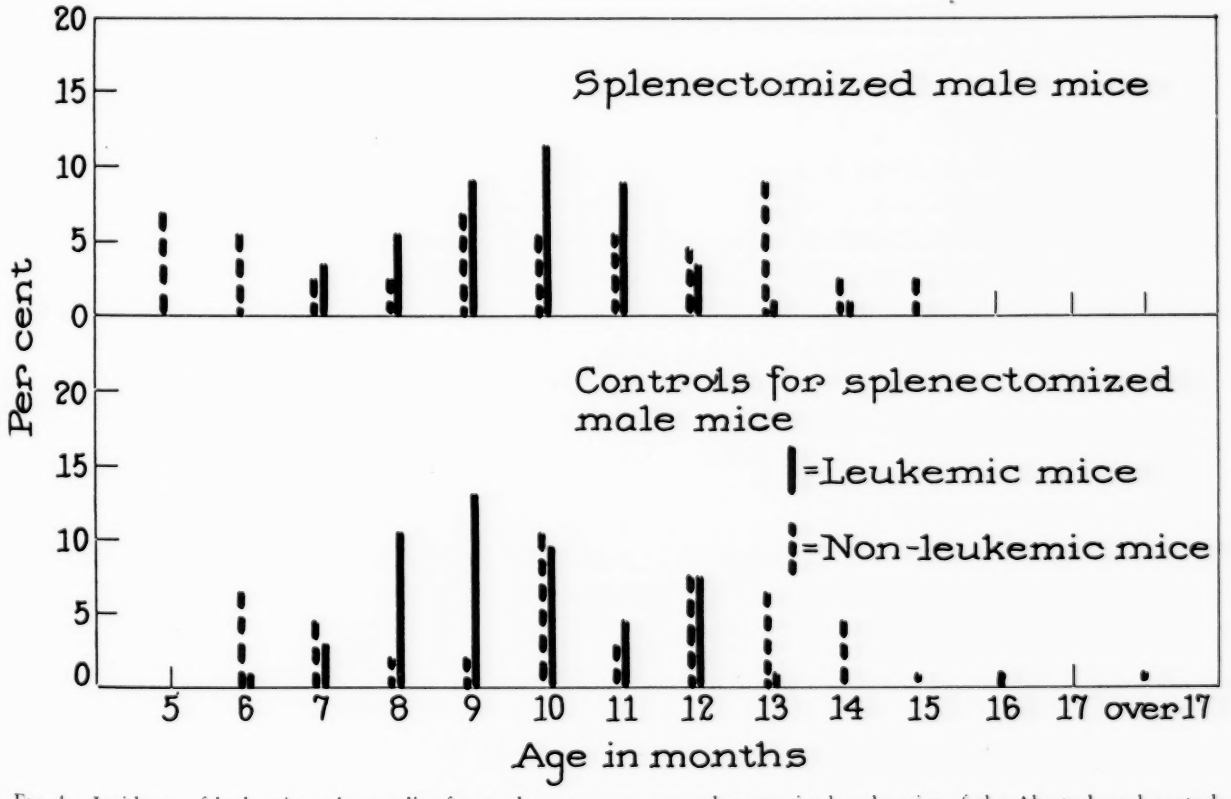

FIG. 4.—Incidence of leukemia and mortality from other causes among splenectomized male mice of the Ak stock and controls.

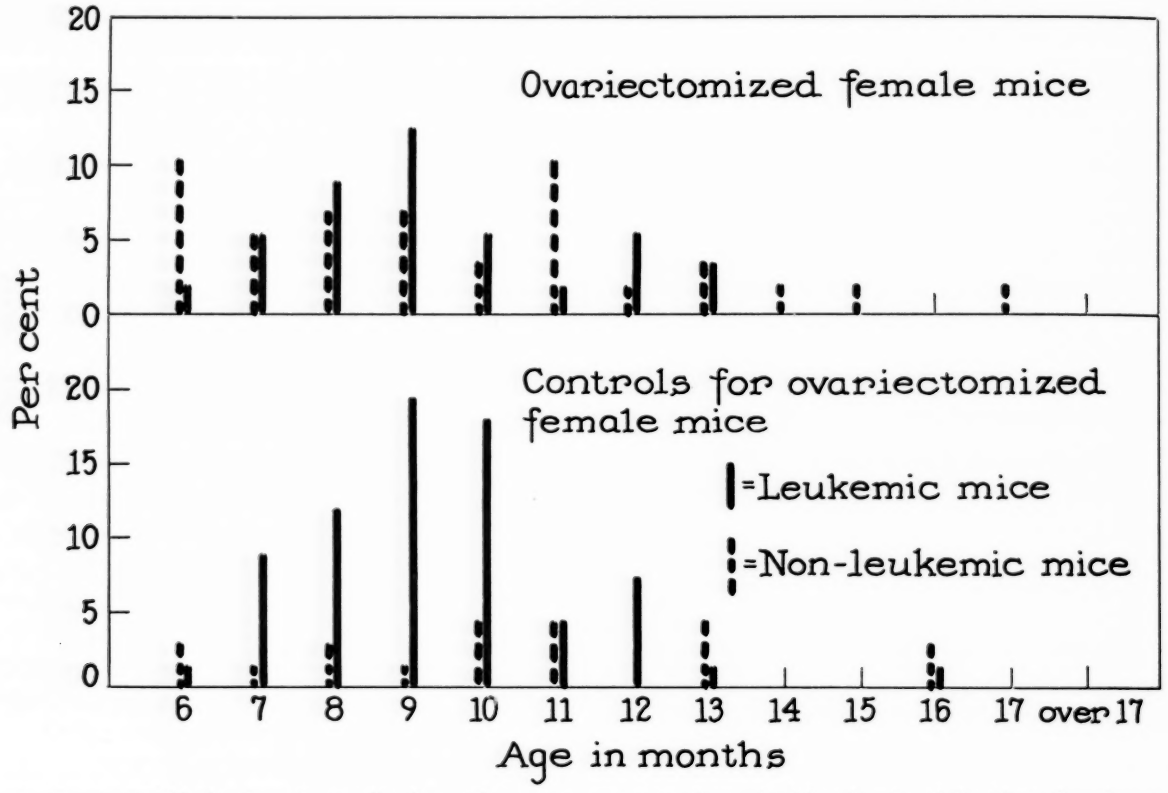

FIG. 5.—Incidence of leukemia and mortality from other causes among ovariectomized female mice of the Ak stock and controls.

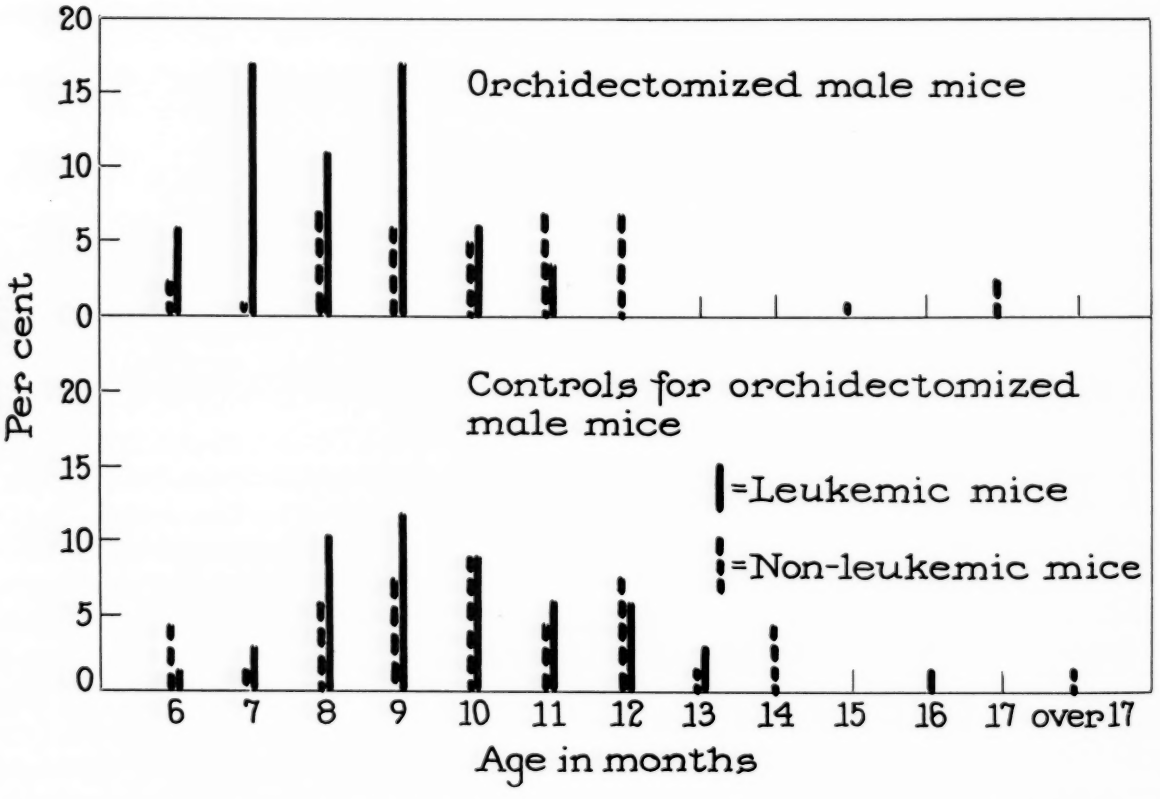

FIG. 6.—Incidence of leukemia and mortality from other causes among orchidectomized male mice of the Ak stock and controls.

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The Effect of Adrenalectomy on the Susceptibility of Rats to a Transplantable Leukemia*

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Transplantable tumors, as a rule, develop more readily in young animals. A transplantable lymphatic leukemia of the rat, which has been under investigation in this laboratory (12), resembles the tumors in this respect. Animals from a susceptible strain, if inoculated under 5 months of age, prove susceptible in something over 95 per cent, while rats between 12 and 15 months of age develop the disease in less than 50 per cent. It would appear, therefore, that the lowered receptivity becomes evident even in the early middle age period, and is certainly acquired before there are definite senile changes. The parent strain (Wistar) from which our rats are derived has an estimated average life span of 33 months, with the menopause occurring between 15 and 18 months of age (4).

The regression of the thymus is the most obvious change taking place with the aging process. According to Donaldson (4) the thymus reaches its largest size in the rat at about 2½ months of age, which corresponds to the beginning of puberty. Thereafter the gland regresses, till at 1 year of age only a small fragment remains. The possibility that the underlying conditions responsible for the thymus regression may be of significance in determining susceptibility to inoculated malignant cells has not received experimental consideration.

Jaffe (10) demonstrated some years ago that removal of the adrenals was followed by regeneration of the thymus in older rats and resulted in a definite stimulation of growth of the gland in young ones. A similar stimulation of the thymus reduced in size by x-ray has been noted by Grégoire (8). In the present study advantage has been taken of this method of thymus stimulation and accompanying reactions following the removal of the adrenals on the natural resistance of old rats and induced resistance of young rats to transplantable lymphatic leukemia.

METHODS

The rats used throughout the following experiments were from a substrain of Wistar rats from which our original leukemia case was derived. Both adrenals were removed with a generous margin of surrounding fat through a dorsal incision. Immediately following the operation each animal was given 2.5 cc. of 1.25 per cent saline solution intraperitoneally and this was repeated daily for 2 to 3 weeks. Throughout the experimental period the drinking fluid provided was Ringer's solution containing 1 per cent lactose and 1 per cent dextrose. Otherwise the diet was the standard one used in this laboratory, consisting of bread soaked in milk with oats and fox chow once a week. This postoperative procedure is a modification of that recommended by other investigators (1, 3, 7, 16).

During the first few days following operation the rats were kept under even temperature conditions. The death rate in the older animals was approximately 16 per cent and no deaths attributable to the adrenalectomy occurred later than 9 days. With the younger group (3 to 6 months) the rate was 20 per cent between the second and 13th days, with no later fatalities due to the operation occurring during the experimental period.

EXPERIMENTS

Group I.—In the four individual experiments making up this group identical procedures were carried out, and the results were so nearly the same that they may be presented together. From 60 rats surviving the immediate effects of adrenalectomy, 39 were inoculated intraperitoneally with 0.2 cc. of leukemic cells 14 to 16 days after the operation. The remaining 21 adrenalectomized animals were not inoculated, and served as survival controls. As control of the activity of the inoculum, 39 intact rats of the same age period were inoculated at the same time as the test rats with 0.2 cc. of leukemic cells. All the rats used were from the same strain, ranged in weight from 250 to 349 grams, and were estimated to be over 15 months of age.

The results of the foregoing experiments are given in Table I and the first three experiments shown graphically in Fig. 1. Of the 39 inoculated adrenal-

* This investigation was aided by a fund for leukemia studies, contributed anonymously.

ectomized rats 89.7 per cent developed the characteristic disease picture of leukemia, manifested by extreme enlargement of the thymus and superficial and mesenteric lymph nodes, increase in circulating lymphocytes, and often involvement of the spleen. The average survival time for the diseased animals was 6.2 days, with the majority dying between the fifth and sixth day and only 1 living as long as 9 days. Of the 39 inoculated intact rats only 43.5 per cent developed leukemia, and these had an average survival time of 9.7 days. Only 1 died as early as 7 days and the majority lived for 11 days. There were no deaths among the uninoculated adrenalectomized rats for the duration of the experiment or for weeks afterwards. The extent of regeneration of the thymus may be judged by comparing the size of the gland in a normal rat in Fig. 2 with that of an adrenalectomized rat of the same age in Fig. 3.

TABLE I: EFFECT OF ADRENALECTOMY ON RESISTANCE OF OLD RATS TO TRANSPLANTED LEUKEMIA

Procedure	Interval	Procedure	Number	Leukemic	Survival time
Adrenalectomy	14-16 days	Inoculation	39	89.7%	6.2 days
Intact rats		Inoculation	39	43.5	9.7 "
Adrenalectomy		No inoculation	21	00.0	All survived

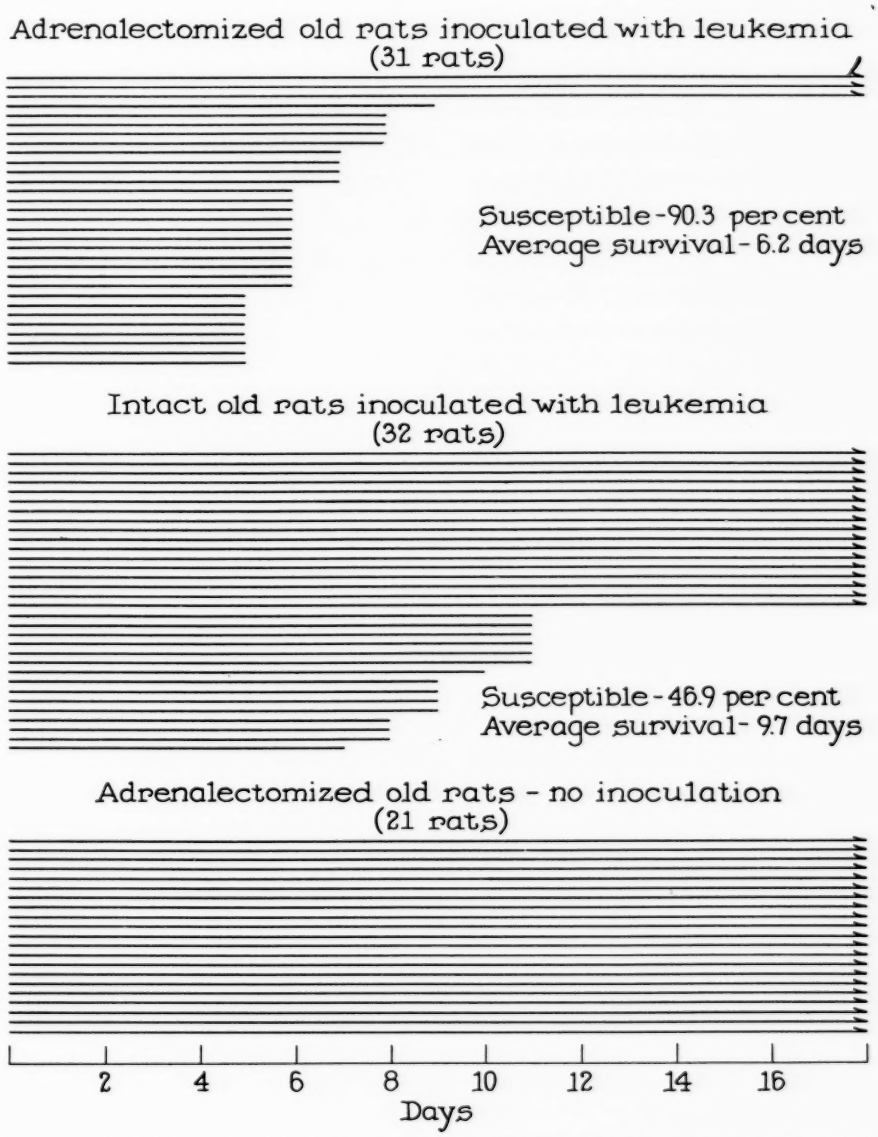
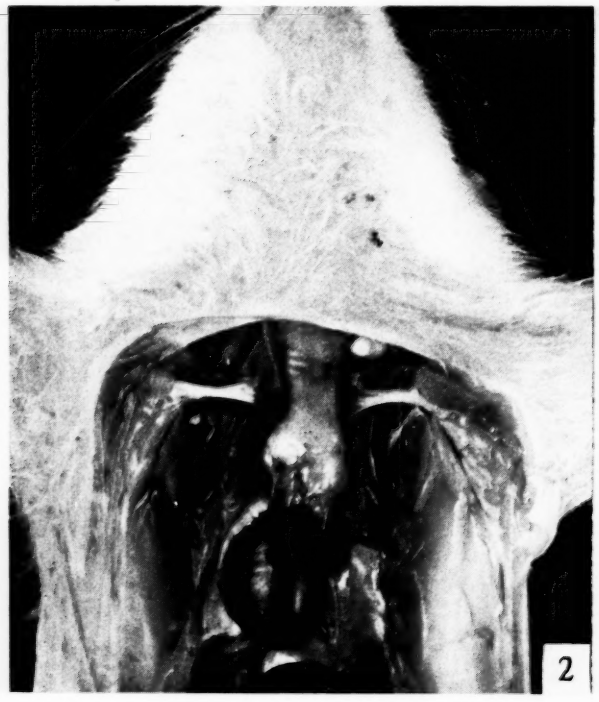


FIG. 1.—Each line represents the time of survival of a rat after inoculation. Those extending to the right edge indicate survival beyond the period at which leukemia would develop.

Group II.—The Rockefeller Institute strain of hooded rats, which normally have a high degree of

resistance to our transplantable leukemia, was used in the next experiment. The adrenals were removed from 11 rats and 2 weeks later they, with 12 intact rats of the same strain, were inoculated intraperitoneally with leukemic cells. All the 11 adrenalectomized rats developed the disease promptly, with an average survival time of only 6.5 days. The control intact rats showed no evidence of the disease.

The foregoing experiments demonstrated clearly that removal of the adrenals reduces the natural resistance of older rats to inoculated lymphatic leukemia. Furthermore, resistance to the disease in at least one refractory strain of rats is completely obliterated by



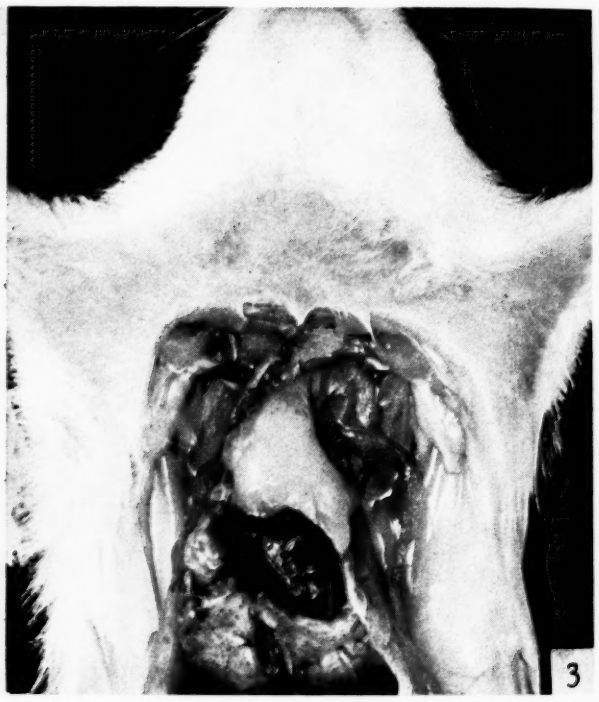


FIG. 2.—Thymus of a normal rat over 1 year old.
FIG. 3.—Thymus of an adrenalectomized rat of the same age as the one shown in Fig. 2.

adrenalectomy. The next experiments were designed to test the effect of adrenalectomy on induced resistance in young rats, a state that develops after an injection of homologous living normal cells some days prior to inoculation. Such a procedure has long been known to induce a degree of resistance to many of the transplantable malignant tumors of animals. Sturm (15) has reported recently that susceptibility to inoculation of our strain of lymphatic leukemia may be also materially reduced by the same method.

Group III.—There were 5 individual experiments in this group, which include observations on a total of 253 young rats. Tests were made of the effect of adrenalectomy on induced resistance to inoculated leukemic cells when the operation was performed at different intervals before and after the immunizing treatment. These results were compared with the degree of susceptibility of immunized intact rats, adrenalectomized nonimmunized rats, and untreated controls. The figures for the various groups, with records of time intervals between the different procedures, are given in Table II. All the rats used in the test were from the same strain, of about the same weight, and were approximately 3 months old. The immunizing dose of 0.5 cc. of defibrinated rat blood from another strain of rats was given intraperitoneally 14 days before the leukemia inoculation. With the exception of one small group, there was an interval of at least 2 weeks between adrenalectomy and inoculation. As noted above, the deaths directly attributable to the adrenalectomy in our control groups occurred before the 14th day. All adrenalectomized animals received the same sustaining postoperative treatment as that used in the first experiment.

The results show clearly that removal of the adrenals definitely interferes with the development of a resistant state, which in intact animals follows the injection of homologous living normal cells. When the glands are removed before the immunizing dose the reduction in potential resistance is perhaps somewhat less than that observed when the immunizing treatment precedes the adrenalectomy. As in the first group, the average survival time of the adrenalectom-

TABLE II: EFFECT OF ADRENALECTOMY ON INDUCED RESISTANCE TO TRANSPLANTED LEUKEMIA

Procedure	Interval	Procedure	Number	Leukemic	Survival time
Adrenalectomy	24-48 hrs.	Immunization	34	76.5%	6.3 days
"	14 days	"	9	88.8	7.1 "
Immunization	24 hrs.	Adrenalectomy	32	90.6	6.6 days
"	14 days	"	10	100.0	6.1 "
Immunization	14 days	Inoculation	59	33.9	9.7 days
Adrenalectomy	2 days	Inoculation	10	100.0	6.4 days
"	14 "	"	23	100.0	6.5 "
"	26 "	"	9	100.0	6.1 "
Adrenalectomy		No inoculation	10		All survived
Controls		Inoculation	57	96.5	8.6 days

ized animals that developed leukemia was significantly shorter than that of the susceptible animals of the other groups.

DISCUSSION

There has accumulated in recent years considerable experimental evidence that hormones play a role in the development and growth of certain types of malignant neoplastic disease. Estrogens have been shown to influence the development of mammary, uterine, and testicular tumors in animals (2, 6, 14) and the male sex hormone appears to stimulate the growth of prostatic cancer in man (9). In some strains cancer of the adrenals frequently develops in mice that have been castrated early in life (18). It is now demonstrated that removal of the adrenals renders a rat susceptible, or more susceptible, to a transplanted lymphatic leukemia. This gives still another indication that interference with the endocrine balance may have an influence on the development of a malignant condition.

It does not necessarily follow that because an induced condition affects susceptibility to transplants of neoplastic cells, such an induced condition may play any part in the origin of a malignant tumor. Yet there is at least one example where this is true. Estrogens may play a role in the experimental production of interstitial cell tumors of the testicle, and in several instances such tumors will grow on transplantation only in mice receiving estrogens (6, 14). While it is not definitely established that the male sex hormone is involved in the origin of prostatic cancer, there is evidence that testosterone stimulates the growth of this type of tumor and that elimination of the male sex hormone by castration, or its neutralization by estrogen, is often followed by notable diminution of growth, particularly of metastases from prostatic cancer in man (9). Considering the fact that the functional activities of certain organs are so completely under the control of the endocrines, it may well be that certain hormones may play some part in the origin and growth of tumors arising in such organs. The indication that lymphoid tissue is at least to a certain extent influenced by the endocrines (5, 13), taken with the present findings that elimination of one of the endocrine glands influences resistance to malignant lymphoid cells, is sufficiently suggestive of a possible hormonal involvement in leukemia to warrant further investigation.

There are a number of effects from removal of the adrenals besides that on the thymus, which must be considered in an analysis of our results. The fact that rejuvenation of the thymus is a prominent feature in the older rats rendered susceptible to leukemia by adrenalectomy cannot be taken as indicative of a relationship between the two conditions. The mere presence of an active thymus in young rats of a resistant strain does not affect their susceptibility, but removal of the adrenals renders these animals 100 per cent susceptible to the disease. This finding does not completely eliminate the thymus changes from consideration, for even in young animals adrenalectomy causes a definite stimulation of the still active gland. It is considered more likely that adrenalectomy results in a condition that stimulates the lymphoid tissue or releases it from control, and that these factors are equally effective in stimulating the development of the inoculated malignant lymphoid cells. If this be the true explanation, the increased susceptibility to leukemia and the stimulation of the thymus would be the result of the action of a common factor with no causal interrelationship.

There is as yet no convincing evidence that the adrenals play any significant role in cancer (11, 17).

SUMMARY

In the experiments reported removal of the adrenals reduced the natural resistance of old rats and the induced resistance of young rats to a transplantable lymphatic leukemia. Inoculation of intact, middle-aged animals of a special strain resulted in 43.5 per cent mortality, while 89.7 per cent of adrenalectom-

ized rats of the same strain and age developed the disease. Young rats with induced resistance gave 33.9 per cent takes following inoculation. Animals in which the adrenals were removed after the resistance-inducing treatment were over 90 per cent susceptible while in another group, adrenalectomized before the immunizing treatment, 78.8 per cent died of leukemia. A different strain of rats, highly resistant to the transplanted leukemia used in the tests, became 100 per cent susceptible following removal of the adrenals.

A prominent feature in the adrenalectomized rats is the regeneration of the retrogressed thymus in old animals and an active stimulation of this gland in young ones. It is suggested that the greater receptivity of adrenalectomized rats to transplanted leukemia is the result of action of the same stimulating factors on the malignant lymphoid cells.

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Abstracts

Experimental Research, Animal Tumors

Carcinogenic Constituents of Shale Oil. Berenblum, I., and Schoental, R. [British Empire Cancer Campaign, Univ. of Oxford, Oxford, England] *Brit. J. Exper. Path.*, **24**:232-239. 1943.

A chromatographic fractionation of Scottish blue shale oil yielded a carcinogenic fraction (a) showing the fluorescence spectrum of 3,4-benzpyrene; oxidation and reductive methylation of this fraction produced the fluorescence bands of a mixture of 5,8- and 5,10-dimethoxy-3,4-benzpyrene, thus providing additional evidence of the presence of the parent compound, which was not isolated; and a more strongly carcinogenic fraction (b) in which the spectrum of 3,4-benzpyrene was absent. By comparison of the fluorescence spectra with those of standard solutions, the authors estimate the amount of 3,4-benzpyrene in crude shale oil at 0.01%, and in coal tar at 1.5%. In the light of experiments with solutions of the pure compound the authors consider that there is not enough benzpyrene in any fraction of shale oil to account for any large part of its carcinogenic power. Probably the unknown carcinogenic compound or compounds would be isolated more easily from coal tar, in which they are present. From coal tar, benzpyrene-free fractions can be obtained that are more strongly carcinogenic than is a saturated solution of benzpyrene in benzene. Chloroform extracts of unheated shale yield no spectroscopic evidence of the presence of benzpyrene; whether they contain other carcinogenic compounds is as yet uncertain.—E. L. K.

Urinary Excretion of Acid-Decomposable Hydrocarbon Precursors Following Administration of Polycyclic Hydrocarbons. Chang, L. H., and Young, L. [Univ. of Toronto, Toronto, Canada] *Proc. Soc. Exper. Biol. & Med.*, **53**:126-129. 1943.

Naphthalene was fed to rats, either dissolved in warm liquid paraffin and given by stomach tube, or mixed with the stock diet to the extent of 1%. As a result, a compound was excreted in the urine, which upon decomposition by acid at pH 1.5 to 2.5 at room temperature yielded naphthalene. Thirteen to 17% of the naphthalene administered was thus recovered. In experiments in which phenanthrene or anthracene was fed, 4% to 7% and 2% to 3%, respectively, were recovered in the urine.

When rats were fed diets containing 1% acenaphthene, chrysene, 3,4-benzpyrene, 1,2,5,6-dibenzanthracene, or methylcholanthrene, no liberation of hydrocarbon was detected in the acidified urine.—M. B.

Problems of Infection. A Lecture Commemorating the Hundredth Anniversary of the Birth of Sir Hector Cameron. Browning, C. H. [Univ. and Western Infirmary, Glasgow, Scotland] *Glasgow M. J.*, **23**:1-21. 1944.

A lecture that includes a discussion of the carcinogenic action of "styryl 430." When injected sub cutem (6 to 10 mgm. in mice, or twice such amounts in rats) in water the compound is precipitated by the serum as deep red granules that are gradually taken up by phagocytes; some diffusion over the rest of the body must take place as the substance is trypanocidal. A minute mass of tissue is formed composed of phagocytes packed with red granules; if a tumor develops, non-pigmented areas of sarcoma cells appear among the phagocytes, and the nuclei of some of the phagocytes enlarge. Amounts of 0.1 mgm. can be detected in the subcutaneous tissues for over a year but do not produce tumors. Peritoneal injection, even of large doses, has given negative results. Persistence of a local deposit is necessary; thus intramuscular injection in fowls, when no deposit is formed, does not produce tumors. In rats, numerous metastases occur. The compound is not estrogenic, and no growth-inhibiting action has been detected. A number of other dyes that form similar persistent deposits, and more soluble styryl compounds, have given negative results; the only other carcinogenic compound discovered has been a close analogue of "styryl 430" which is likewise very little soluble in serum. The tumors are not filtrable and are not propagated better by finely ground material than by ordinary grafts (Peacock).—E. L. K.

Fluorescent Concentrates from the Nonsaponifiable Fractions of Human Livers. Jones, R. N., and May, C. D. [Queen's Univ., Kingston, Ontario; and Harvard Univ., Cambridge, Mass.] *Cancer Research*, **4**:304-312. 1944.

Three procedures are described for the preparation, from the nonsaponifiable fraction of human livers, of neutral oils exhibiting intense blue or bluish-green fluorescence. Concentrates of a grossly similar character have been obtained from healthy livers, livers of cancer patients showing no liver metastases, and the liver of a patient with lymphosarcoma and hepatic necrosis. The concentrates obtained by the 3 different methods all have ultraviolet absorption spectra with a maximum or plateau at 2,550–2,600 A., but neither the ultraviolet nor the fluorescence spectra show the fine-banded structure of the type associated with polynuclear aromatic hydrocarbons. The quenching effect of oxygen on the intensity of fluorescence is less than that usually observed for solutions of such hydrocarbons.

The fluorescing component of the concentrate exhibits considerable chemical stability. It is not affected by *N* aqueous hydrochloric acid or by alkali, nor does it react with reagents that attack the hydroxyl group (succinic

anhydride, pyridine-sulfur trioxide) or with digitonin, Girard's reagent, maleic anhydride, or an aqueous solution of sodium hydrosulfite. It appears to be adsorbed specifically on picric acid from an ethanolic solution. Although the spectrographic data do not encourage the view that the fluorescing substance is a polynuclear aromatic hydrocarbon, it has been demonstrated that methylcholanthrene, when added to liver tissue, accumulates in the same fraction.—Authors' abstract.

Effects on Rats of Prolonged Feeding with the Staple African Diet. GILLMAN, J. [Univ. of the Witwatersrand, Johannesburg, So. Africa] *Brit. M. J.*, **1**:149-150. 1944.

Berman and others have drawn attention to the high incidence of cirrhosis, and primary cancer, of the liver in the Bantu of South Africa (abstracted in *Cancer Research*, **1**:176-177, 915. 1941; **2**:591. 1942.) The question arises, whether these conditions are due to racial or environmental factors. "Malnutrition among the Africans is widespread in South Africa. The overwhelming majority feed on a diet consisting largely of maize meal (mealie pap) and sour milk. This forms the staple from the time of weaning throughout life." One hundred and twenty-five rats (40 to 50 gm. weight) were fed solely on mealie-meal porridge and sour milk. No obvious signs of avitaminosis were seen. After 14 months 12 rats were killed, these showed: (a) in the liver either enlargement and fatty change or various degrees of nodular cirrhosis; in one case the left lobe was reduced to 2 small cirrhotic nodules while the right lobe was greatly enlarged; (b) enlargement of heart; (c) thickening of skull; (d) fracture and loosening of upper incisors; (e) enlargement of pituitary.

No such changes in the liver had been seen in several thousand other rats. No tumors are mentioned.—E. L. K.

Lactation Activity, Chemical Composition, and *in vitro* Metabolism of Rat Mammary Tissue. KLEIBER, M., SMITH, A. H., and LEVY, P. [Univ. of Calif., Davis, Calif.] *Proc. Soc. Exper. Biol. & Med.*, **53**:94-96. 1943.

Mammary tissue of rats (Long-Evans strain) was analyzed, at the end of pregnancy and at the height of lactation, for dry matter and nitrogen content, and its metabolic rate was measured *in vitro*. It was found that lactation increased the water content of the mammary tissue and the protein content of the mammary dry matter. Lactation did not affect the metabolic rate per unit of fresh tissue *in vitro;* it increased the metabolic rate per unit of dry matter and decreased the metabolic rate per unit of nitrogen in the tissue.—M. B.

Production of Malignancy in Vitro. VII. Metabolism and Biotin Vitamer Content of Tumors Produced. BURK, D., EARLE, W. R., and WINZLER, R. J. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **4**:363-372. 1944.

Seven strains of transplanted sarcomas, derived from mouse subcutaneous fibroblasts that had been subjected to various dosages of 20-methylcholanthrene in tissue culture, were found to possess a metabolism highly characteristic of malignant tumors generally, with respect to all criteria tested, including anaerobic glycolysis, aerobic glycolysis, respiratory quotient, oxygen consumption, oxidation reserve (increase in oxygen consumption on addition of succinate or paraphenylenediamine), Pasteur effect, Meyerhof quotient, extent quotient, and fermentation excess. The biotin and miotin contents of these tumors of *in vitro* origin were likewise typical of values found in malignant tumors of *in vivo* origin, whether spontaneous or induced.

The metabolism of the 7 strains of tumors was qualitatively and quantitatively very similar. Analysis of the data indicated, however, a rough, second-order correlation between minor differences in the anaerobic glycolysis values of the tumors and previously reported differences in growth rates and inoculation responses of the original tissue cultural strains. Lowering the pH of the medium containing the tumor tissue slices caused a considerably greater decrease in anaerobic glycolysis in the case of the 3 least altered strains, HW, D, and H, as compared with the 4 most altered tumor strains, J, L, N, and O. This suggests that a more specific correlation between degree of malignancy and ability to glycolyze might be obtained at pH values considerably below that of blood, but closer to those that probably prevail within the tumor intercellularly.—Authors' summary.

Effect of Amino Acids on the Induction of Leukemia in Mice. WHITE, J., MIDER, G. B., and HESTON, W. E. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **4**:409-411. 1944.

Strain dba mice painted with methylcholanthrene developed essentially the same incidence of leukemia on diets with or without adequate lysine. The data suggest that cystine played a role in the development of induced leukemia, perhaps not associated with its properties as an essential amino acid for growth, but with some other undetermined property. The addition of gelatin to a low cystine diet altered the occurrence of leukemia following methylcholanthrene painting from 10 to 32%. This increase was attributed to the small amount of cystine present in gelatin. The incidence of leukemia in methylcholanthrene-painted mice was increased to the same extent when the low cystine diet was supplemented with methionine as with cystine.—Authors' summary.

Incidence of Leukemia in Physicians. HENSHAW, P. S., and HAWKINS, J. W. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **4**:339-346. 1944.

A comparison was made of the incidence of leukemia in physicians and in the general population. Data were obtained from the death lists of the Journal of the American Medical Association, from the mortality reports of the United States Bureau of the Census, and an unpublished compilation of the United States Public Health Service. Comparisons were made on the basis of (1) the ratio of deaths from leukemia to deaths from cancer, (2) the ratio of deaths from leukemia to total death rates, and (3) death rates from leukemia.

Leukemia was recognized approximately 1.7 times more frequently among physicians than among white males in the general population. Possible discrepancies in the data are discussed. While these observations furnish no direct proof that radiation acts to incite leukemia in human beings, they are, nevertheless, in accord with the findings on experimental animals in which exposure to

x-rays has been found to increase the incidence of leukemia.—Authors' summary.

Influence of Environmental Temperature upon the Incidence and Course of Spontaneous Tumors in C3H Mice. WALLACE, E. W., WALLACE, H., and MILLS, C. A. [Univ. of Cincinnati, Cincinnati, Ohio] *Cancer Research,* **4**:279-281. 1944.

Spontaneous mammary cancer in virgin C3H female mice showed the same increased incidence in cool environments that was previously found in virgin dba females under these conditions. The tumors appeared 1 month earlier and grew faster at 68° F., although they killed the hot-room mice more quickly. In this C3H strain series multiple tumors were 4 times more frequent among mice in the cold-room than among those kept in the heat.—Authors' summary.

Chemical Structure and Antifibromatogenic Activity of Steroid Hormones. LIPSCHÜTZ, A. [National Health Service of Chile, Santiago, Chile] *Nature,* **153**:260-261. 1944.

The production of fibromas in female guinea pigs by estrogens (Lipschütz, A., and Iglesias, R. *Compt. rend. Soc. de biol.,* **129**:519-524. 1938) is prevented by simultaneous administration of certain steroids (the naturally occurring progesterone, desoxycorticosterone, dehydrocorticosterone, and testosterone; and dihydrotestosterone, which has not been found in the body). All these 5 compounds are 3-keto-steroids; the last-named lacks the double bond Δ^4 which is therefore not essential. Two other compounds lacking this double bond (pregnanedione and allopregnanedione), but having the same side-chain at C_{17} as progesterone, were inactive, as was also Δ^{16} dehydroprogesterone, which differs from progesterone by a double bond in ring IV. The activity of testosterone was abolished by oxidation at C_{17} (Δ^4-androstene-3-17-dione) or by elongation of the side-chain (cholestenone). Compounds having hydroxyl at C_3 (acetoxypregnenolone, androsterone, androstanediol, androstenediol) were inactive. All antifibromatogenic 3-keto-steroids are to a varying extent progestational, and no inactive 3-keto-steroid, with the possible exception of androstenedione, is known to be progestational. There is no uniform relation between antifibromatogenic, and either masculinizing or antiestrogenic, activity.—E. L. K.

Experimental Brain Tumors. V. Behavior in Intraocular Transplants. FREEMAN, D., and ZIMMERMAN, H. M. [Yale Univ. Sch. of Med., New Haven, Conn.] *Cancer Research,* **4**:273-278. 1944.

A technic is described for mice and guinea pigs that permits the intraocular transplantation of brain tumors, both those induced with a chemical carcinogen in mice and those occurring spontaneously in man. This method of study affords the opportunity of keeping the growing neoplasms under constant observation. It has demonstrated certain characteristics of neoplastic growth behavior and appearance that permit differentiation of gliomas from nongliogenous tumors. It has demonstrated, by the standard of autonomous growth in homologous and heterologous strains of mice, that experimentally induced brain tumors represent true neoplasms.—Authors' summary.

Studies on Melanoma. SUGIURA, K. [Memorial Hosp., New York, N. Y.] *Cancer Research,* **4**:282-288. 1944.

The following conclusions are drawn concerning the Harding-Passey mouse melanoma: (1) Variations in the age of the host did not influence the outcome of transplantation. (2) Suckling mice offered a favorable soil for the continued growth of the melanoma. (3) The survival time after grafting was the same for suckling mice and for adults. (4) Castration in males and females did not significantly affect the growth of the neoplasm. (5) The growth capacity of the melanoma was completely destroyed by immersion in a buffer solution at pH 4 or 10, and at pH 5 or 9 partial inhibition and delayed growth were found. No effect was observable at pH 6, 7, or 8. (6) The viability of the melanoma was completely destroyed by dehydration from the frozen state. (7) The tumor was not filtrable. (8) Grafts grew equally well in C57 black, dba, C3H, agouti, Rockland, Bagg, Swiss, and Paris albino mice. (9) During the past 15 years the tumor has been propagated through many generations by successive graftings. So far no nonpigmented melanoma has appeared.—Author's abstract.

The Relation to Chick Tissues of Tumors Produced by the Yolk Injection Technic. HUNGATE, R. E., TAYLOR, A., and THOMPSON, R. C. [Univ. of Texas, Biochemical Inst., and Clayton Foundation for Research, Austin, Texas] *Cancer Research,* **4**:289-292. 1944.

The following observations have been made: Tumor cells introduced into chick embryos by the yolk injection technic become implanted on the mesoderm outside of the endoderm. This is possible because at the time of injection the yolk is not yet enclosed by endoderm. The endoderm grows under some of the injected cells, leaving them in contact with the developing mesoderm, which furnishes the blood supply necessary for further growth. Implantation can occur only before the endoderm encloses the yolk. Experience has shown that the 4 day egg is the most favorable for injection. The most successful implantation is obtained by forcible injection of tumor tissue and by frequent rotation of the egg.—Authors' abstract.

The Effect of Mercury-Indigo-Disulphonate on Breast Cancer of Mice. DAVIS, J. E. [Biochemical Research Labs., Chicago, Ill.] *Canad. M. A. J.,* **48**:443-444. 1943.

Repeated injection of a solution of mercury-indigo-disulphonate directly into mammary tumors of 40 mice was followed by disappearance of the growths in 30 animals and by regressing of the tumors in the remaining 10. No case of retrogression or disappearance of cancer was noted in the control mice. (The number of mice and the treatment in the controls was not stated.)—A. C.

Is Cancer a Communicable Disease? GROSS, L. [U. S. Army] *Cancer Research,* **4**:293-303. 1944.

Recent experiments demonstrate clearly that mammary cancer in mice is communicable from one generation to another. Animals transmitting the disease are, as a rule, carriers of a latent tumor factor and do not themselves display symptoms until they reach the "tumor age." The development of mammary cancer can be entirely avoided in susceptible mice by preventing newly born animals from nursing their potentially cancerous mothers.

The available data on accidental or intentional inoculation of human cancer are reviewed, and the appearance of tumors in several members of the same or successive generations in man is discussed. The conclusion is suggested that human cancer may be similar to that observed in mice and may also, perhaps, be communicable from one generation to another.

Since milk seems mainly responsible for the transmission of certain tumors such as mammary carcinoma, it is suggested that the women of families with any malignant tumors in their ancestry refrain entirely from nursing their progeny. Artificial feeding should be substituted *from birth,* at least for one generation. This simple preventive measure may bring substantial rewards in the fight against cancer, although results will not become evident until the next generation reaches the tumor age.—Author's summary.

Chemistry and Cancer. Cook, J. W. [Univ. of Glasgow, Glasgow, Scotland] Royal Inst. of Chemistry of Great Britain and Ireland. 1943.

A lecture.—E. L. K.

Clinical and Pathological Reports

Etiology

Syphilis and Cancer. Reported Syphilis Prevalence Among 7,761 Cancer Patients. Levin, M. L., Kress, L. C., and Goldstein, H. [N. Y. State Dept. of Health, Albany, N. Y.] *New York State M. J.,* **42**:1737-1745. 1942.

The prevalence of syphilis in a sample of 7,761 cancer patients reported in upstate New York in 1940–1941 was determined by comparing cancer case reports with the registers of reported syphilis cases. Of 3,151 white male cancer patients 3.2% were found also to have been reported as syphilitic. Of 4,610 white female cancer patients 1.7% were found to have syphilis. Syphilis prevalence among males with tongue cancer and females with cervix cancer was significantly greater than in patients with cancer of other sites.

On the basis of the present findings, special efforts are indicated toward early discovery of cervix cancer in women who have had syphilis.—J. L. M.

Radiation—Diagnosis and Therapy

The Similarity of Clinical and Roentgen Findings in Children with Ewing's Sarcoma (Endothelial Myeloma) and Sympathetic Neuroblastoma. Barden, R. P. [Hosp. of Protestant Episcopal Church, Philadelphia, Pa.] *Am. J. Roentgenol.,* **50**:575-581. 1943.

Ewing's sarcoma and sympathetic neuroblastoma present definite similarity in histopathology and radiographic appearance. Case histories are given of 4 children with widespread bone tumors, 2 of whom died of the former, and 2 of the latter disease. The differential diagnosis in each case could be made only at postmortem examination.

The author feels that cases of solitary bone tumor diagnosed as Ewing's sarcoma should be treated as if the bone tumor were secondary to an abdominal tumor whether the presence of the latter can be established or not. Amputation for cure should probably not be attempted, and x-ray treatment to retroperitoneal structures should be routine.—E. H. Q.

The Problem of Recovery from Radiation Effects. Ellinger, F. [Long Island Coll. of Med., Brooklyn, N. Y.] *Radiology,* **40**:62-71. 1943.

The biologic, photochemical, and clinical aspects of tissue recovery from radiation are discussed, and a distinction is made between "true recovery" and "pseudo-recovery." Most tissues exhibit pseudo-recovery, which is restoration due to growth of cells uninjured by radiation rather than true recovery of single cells.—R. E. S.

A Consideration of the Response of Bladder Tumors to External Radiation. Herger, C. C., and Sauer, H. R. [State Inst. for the Study of Malignant Diseases, Buffalo, N. Y.] *J. Urol.,* **50**:310-321. 1943.

The problem of tumor radiosensitivity is outlined, principally according to the theories of Stewart and Warren. Of 160 cases of carcinoma of the bladder treated by x-radiation 14, or 8.8%, had complete disappearance of the lesion (4 of these, however, had a reappearance of the neoplasm). Marked regression was obtained in 51.9%, and no apparent regression in 39.3%. The solid infiltrating carcinomas responded very poorly, but over 50% of the papillary type showed satisfactory results.—V. F. M.

Noncarcinomatous Postirradiation Ulcerations of the Cervix. Jacox, H. W., Johnston, J. R., and Gross, P. [Western Pennsylvania Hosp. Tumor Clinic and Inst. of Pathology, Pittsburgh, Pa.] *Pennsylvania M. J.,* **46**:119-121. 1942-43.

Following radiation therapy of carcinoma of the cervix, radiation necrosis may occur not only in the bladder and rectum but at or near the site of the cervix itself. These lesions may arise many months after the original carcinoma has disappeared and the radiation reaction has subsided. Such lesions may suggest recurrence of the tumor, and they necessitate biopsy to establish the correct diagnosis and to avoid improper treatment. Four illustrative cases are described.—J. L. M.

The Treatment of Congenital Hemangiomata of the Skin. Johnson, G. S., and Light, R. A. [Vanderbilt Univ. Sch. of Med., Nashville, Tenn.] *Ann. Surg.,* **117**:134-139. 1943.

Seven cases of hemangioma were treated with implantation of radon. Each seed (0.25 to 1.0 mc.) was used to irradiate approximately 1 cc. of tissue. Photographs after this procedure are given as evidence favoring its use where cosmetic results are important and surgery and scarring are undesirable.—W. J. B.

The Classification of Laryngeal Cancer from a Radiotherapeutic Viewpoint. Lederman, M. [Royal Cancer Hosp. (Free), London, England] *Brit. J. Radiol.,* **16**:298-300. 1943.

The subdivision of laryngeal cancer into intrinsic and extrinsic forms is much less satisfactory to the radio-

therapist than to the surgeon. The following classification is suggested as a means of helping the radiotherapist to deal with his special problems.

A. The term cancer of the larynx should be limited to the intrinsic forms found in the following sites: 1) True vocal cord. 2) Ventricle, false cord, and infrahyoid epiglottis. 3) Subglottic space. 4) Commissure, anterior and posterior. It is important to subdivide the epiglottis into supra- and infrahyoid parts.

B. The term extrinsic cancer of the larynx should be abandoned, and a laryngopharyngeal group of neoplasms recognized and subdivided as follows:

1) Epilaryngeal group arising in relation to the margin of the laryngeal vestibule, *i.e.*, (a) Suprahyoid epiglottis. (b) Aryepiglottic fold. (c) Arytenoids.

2) Epiesophageal group in relation to walls of the pharyngeal channel leading to the esophagus, *i.e.*, (a) Post cricoid. (b) Pyriform fossa. (c) Lateral and posterior walls of laryngopharynx.

The objects and advantages of the proposed classification are as follows: 1) The neoplasms are grouped clinically and according to response to radiotherapy. 2) Selection of treatment method and technic is facilitated. 3) A basis is provided for prognosis and comparison of results of differing methods of treatment obtained at different centers.—W. V. M.

The Technique of Radium Treatment of Intrinsic Cancer of the Larynx. Lederman, M., and Mayneord, W. V. [Royal Cancer Hosp. (Free), London, England] *Brit. J. Radiol.*, **16**:301-307. 1943.

The paper consists of a discussion of a number of the more technical aspects of the treatment of cancer of the larynx by teleradium. It is insisted that ability to individualize treatment is of paramount importance in radiotherapy, and that radiation should be administered according to the needs of the particular patient, the responses of the patient being the basic guide to such needs. The 4 main topographical types of tumor in the larynx, namely, those involving the anterior half, the posterior half, the centre, and finally, one lateral half of the larynx, lead to a discussion of the necessity for different technics of treatment. Eight teleradium technics have been analyzed with some care, complete distributions of radiation in two mutually perpendicular planes being calculated by use of dose finder and contour projector. From a study of postmortem specimens, anatomical charts and models have been constructed, and the distribution is shown in relation to these charts. The advantages of different technics in the treatment of each of the topographical sites are discussed in detail. For comparison with these teleradium technics the Finzi-Harmer fenestration technic has been studied. Of 15 patients treated over 5 years ago, in no less than 9 the disease has been eradicated, and 7 are alive and free from disease.—W. V. M.

A Correlation of Roentgenogram and Pathological Changes in Ossifying and Chondrifying Primary Osteogenic Neoplasms. Luck, J. V. [State Univ. of Iowa Hosps., Iowa City, Iowa] *Radiology*, **40**:253-276. 1943.

The discussion is limited to those osteogenic neoplasms that consistently produce bone or cartilage. The differential diagnosis of bone tumors calls for close co-operation between the roentgenologist, pathologist, and clinician.—R. E. S.

Diseases of the Mediastinum and Associated Conditions. A Refresher Course. Paul, L. W. [Univ. of Wisconsin Med. Sch. and State of Wisconsin Gen. Hosp., Madison, Wis.] *Radiology*, **40**:10-41. 1943.

This is a comprehensive discussion, from the roentgenological viewpoint, of diseases of the mediastinum classified according to the anatomical structures involved. It includes diseases of lymph nodes; primary tumors of the mediastinum other than lymph node tumors; mediastinitis; diseases of the thyroid, thymus, spine, esophagus and stomach; cardiovascular lesions; and bronchiogenic carcinoma.—R. E. S.

Skin and Subcutaneous Tissues

Congenital Sarcoma of Extremities. Report of Two Cases. Chamberlain, J. W., and Lawrence, K. B. [Massachusetts Memorial Hosps., and Children's Hosp., Boston, Mass.] *Am. J. Dis. Child.*, **62**:793-800. 1941.

Both children are living and well 27 and 20 months, respectively, after amputation of the affected limb.—C. J. M.

Carcinoma of the Skin. Statistical Analysis of 560 Basal Cell Carcinomas. Sutton, R. L., Jr. [Univ. of Kansas Med. Sch., Kansas City, Kan.] *J. Missouri M. A.*, **39**:203-207. 1942.

Histologic diagnoses under 36 classifications included all of 1,500 specimens that clinically were thought to be tumors of the skin. There were 560 basal cell carcinomas. The incidence of this type of tumor was approximately equal in the two sexes. The anatomical distribution was as follows: on the face, 82%; head, 89%; head and neck together, 96%; trunk, 3%; extremities, 1.4%. No tumor occurred on the foot, digits, or the genital or oral mucosal regions. Although basal cell carcinomas comprised only 37% of all lesions in the series, 77% of all lesions removed from the eyelid, 75% from the upper lip, 68% from the nose, and 53% from the chin were basal cell carcinomas. The proportion of all lesions from the temple, ear, and hand that were basal cell carcinomas was apparently significantly higher in males than in females. This relation was reversed for lesions of the forehead, upper lip, and trunk. In other locations the proportions were not significantly different.—J. L. M.

Congenital Melanoma. Report of a Case in which Antenatal Metastasis Occurred. Sweet, L. K., and Connerty, H. V. [Gallinger Municipal Hosp. and Georgetown Univ. Sch. of Med., Washington, D. C.] *Am. J. Dis. Child.*, **62**:1029-1041. 1941.

The body of the child at birth was covered with numerous, darkly-pigmented, elevated lesions. At necropsy, 17 days later, the diagnosis favored was congenital melanotic nevi with antenatal metastatic non-pigmented melanoma in skin, liver, and brain. The paper includes a short review of the literature.—C. J. M.

Adenoid Cystic Basal Cell Carcinoma. Yates, E. C. [Lexington Clinic, Lexington, Ky.] *Kentucky M. J.*, **40**:457-461. 1942.

Two case reports are presented, one in which general metastases occurred from a tumor of the nose, and the

other in which the tumor was localized in the ear with a palpable node in the neck.—J. L. M.

Radical Surgery for Carcinoma of the Skin. Young, F. [Univ. of Rochester Sch. of Med. and Dent., Rochester, N. Y.] *New York State J. Med.*, **43**:836-842. 1943.

The following groups of skin cancer can be arrested in a high percentage of cases by wide, deep surgical removal and immediate surgical repair: skin cancers occurring in burn and postradiation scars, those that are extensively ulcerated, postradiation recurrences of skin cancer, persistent recurrent skin cancer, and skin cancer that has invaded bone. The accepted teaching that cancer defects should be left open for inspection for at least 18 months causes a long period of disability that is not always necessary. Deep invasive growths whose complete eradication is doubtful, should not be repaired until it is certain that recurrence is not probable. However, large surface areas of growth should not be a deterrent to immediate repair.—J. L. M.

Nervous System

Supratentorial Blood Vessel Tumors with Cyst Formation. Cohen, I. [Mt. Sinai Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.*, **9**:354-362. 1942.

Eight cases of tumors of blood vessel origin lying above the tentorium are added to the 2 previously reported by the author. All the tumors were associated with cyst formation. The 10 patients, with one exception, were between the ages of 20 and 40 years; they presented clinical signs in accord with the location of the tumors. The history was short, and the progression of the disease was rapid, in all but 2 cases. Two patients died after craniotomy; 7 of the remaining 8 are known to be alive, the survival period ranging to 9 years. The author believes that in general the prognosis for these patients is good.—S. A. G.

Behavior Disorders Associated with Intra-Cranial Tumors in Childhood: Report of Cases. Langford, W. S., and Klingman, W. O. [Babies Hosp. and Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.] *Am. J. Dis. Child.*, **63**:433-452. 1942.

Report of 3 cases with a psychiatric study.—C. J. M.

Trigeminal Neuralgia and Tumors of the Gasserian Ganglion. Love, J. G., and Woltman, H. W. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **17**:490-496. 1942.

Two cases are reported of trigeminal neuralgia in which an unsuspected tumor involving the gasserian ganglion was found in the course of section of the posterior root. These were the only such cases that had been found by the authors, in spite of the large number of cases of trigeminal neuralgia seen by one of them (Love) in surgical consultation. Only one similar case was found in the records of the Mayo Clinic.—J. L. M.

A Case of Neurofibromatosis in a Child 5¾ Years of Age. Schick, B. [The Mt. Sinai Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.*, **10**:399-401. 1943.

A child of 5¾ years presented a tumor in the upper right chest and mediastinum. After exploratory thoracotomy a diagnosis of neurofibroma was made. The mass was too fixed for safe removal, and radiotherapy was instituted. Five years later the patient showed a distinct Horner syndrome on the right side, and a cervical neurofibroma was excised. Characteristic areas of brown macular pigment also were present over the skin. After 6 years, the general condition is entirely satisfactory, and there are no new neurofibromas.—A. Cnl.

Female Genital Tract

Ovarian Cysts Complicating Pregnancy. Capone, A. J. [Somerville Hosp., Somerville, Mass.] *Am. J. Surg.*, **61**:387-393. 1943.

A discussion and presentation of 3 cases.—W. A. B.

Quantitative Pregnancy Tests in the Diagnosis of Hydatid Mole and Chorionepithelioma. Frank, R. T. [Mt. Sinai Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.*, **10**:112-118. 1943.

A positive pregnancy test signifies a pregnancy, including ectopic gestation, or the presence of chorionepithelioma arising from pregnancy or teratoma. In very rare instances, other tumors, after widespread metastases have developed, can give a typical pregnancy test. The increase in gonadotropic factors, upon which pregnancy tests are based, reaches a tremendous peak between the 30th and 50th day in normal pregnancy. In hydatid mole and chorionepithelioma, irrespective of origin, the gonadotropic factors are elevated above the level reached in normal pregnancy except that attained during the peak period. Therefore, the quantitative pregnancy test is of value only if the peak is kept in mind. This of course does not apply to a postive test in the male, in whom no peak occurs. A low estrogen blood or urinary titer in the female after the 17th week, gives confirmatory evidence of the presence of a pathologic gestational condition (either death of the fetus, or presence of chorionepithelioma, or hydatid mole).—A. Cnl.

Intestinal Obstruction in a Case of Endometriosis. Harte, M. S. [Mt. Sinai Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.*, **10**:292-293. 1943.

The most common extra-abdominal site for endometriosis is the rectovaginal septum. If untreated, the lesion is prone to extend to the adjacent sigmoid and rectum, where continued progression may produce symptoms of intestinal obstruction in the same way that carcinoma does in this location. Treatment may be by means of surgery, x-ray, radium, or any combination of these. Excision of the lesion is preferable in the younger age groups, if feasible. Cessation of activity in the lesion with gradual cure results from castration, either radiotherapeutic or surgical. A case of endometriosis of limited extent is reported in which treatment was adequate but complicated by pelvic abscess and inflammation producing intestinal obstruction. The roentgen features simulated carcinoma of the large intestine. Hemorrhage from the large bowel resulted from ulceration, and a rectovaginal fistula also appeared. Drainage of the pelvic abcess and colostomy resulted in subsidence of the clinical manifestations and progress toward cure.—A. Cnl.

Fibroids in Pregnancy, Labor and Puerperium. LAZARD, E. M. [Los Angeles, Calif.] *West. J. Surg.*, **51**:119-122. 1943.

Cases of pregnant women with tumors above the pelvic brim should be treated expectantly and should be carefully supervised. Surgical interference in pregnancy is practically always necessitated by the effects of pregnancy on the fibroids and frequently may be done without disturbing the pregnancy.—M. E. H.

Benign and Malignant Testicular Tubular Adenoma. Report of Three Cases. NOVAK, J. [Mt. Sinai Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.*, **10**:250-268. 1943.

Tubular testicular adenoma is not only one of the rarest, but also one of the most peculiar, ovarian tumors. Its significance in pathology is based on the fact that it fills the gap between true hermaphroditism and ovarian tumors of male character and opens the way for the understanding of the origin of these interesting neoplasms. Two cases of benign testicular adenoma occurring in 2 sisters are reported. The gonads consisted preponderantly of testicular tissue, but the patients looked and felt perfectly feminine and had female external genitals. Removal of the malformed gonads caused severe deficiency symptoms, thus suggesting a hormonal activity of these gonads. Several other members of the family were intersexual individuals. A third case is reported in which a carcinomatous testicular tubular tumor occurred in a previously normal woman. In this case, as in other reported cases, no hormonal activity of the tumor could be proved. The gonads in the 3 cases are interpreted as ovotestes and the architecture of ovotestis is explained on the basis of embryologic facts. Goldschmidt's intersexuality theory is applied to explain the origin of the ovotestes.—A. Cnl.

The Demonstration of Malignant Cells in Vaginal Smears and Its Relation to the Diagnosis of Carcinoma of the Uterus. PAPANICOLAOU, G. N., and TRAUT, H. F. [Cornell Univ. Med. Coll., New York, N. Y.] *New York State J. Med.*, **43**:767-768. 1943.

For the past 2 years vaginal smears have been made routinely upon every woman admitted to the gynecologic service of the New York Hospital. By the use of the vaginal smear a considerable number of asymptomatic and therefore unsuspected cases of malignant uterine growths have been discovered, some of them in such an early stage of development that they were invisible to the unaided eye or undemonstrable by the biopsy method. Two illustrative cases, one of adenocarcinoma of the fundus and the other of squamous carcinoma of the cervix, are reported. Criteria are given for the diagnosis by this method of squamous carcinoma and adenocarcinoma of the cervix; adenoacanthoma, adeno malignum, and adenocarcinoma of the fundus. Semiannual examination by the vaginal smear method for every woman in the cancer-bearing period of life is urged.—J. L. M.

Hemorrhagic Ovarian Cysts and Menometrorrhagia Accompanying Thrombocytopenic Purpura Hemorrhagica and Necessitating Hysterectomy in a Young Woman. RUBIN, I. C. [Mt. Sinai Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.*, **10**:673-677. 1944.

The case is unique in that large hemorrhagic ovarian cysts with menometrorrhagia developed before pathognomonic evidence of the underlying purpuric state was adduced. The characteristic picture of purpura hemorrhagica presented itself only after the surgical procedure that was resorted to in order to correct the uterine bleeding. The importance of searching carefully for symptoms suggesting a possible hemorrhagic diathesis in instances of gynecologic lesions associated with menometrorrhagia in young women is pointed out. Complete and thorough blood examinations are essential despite the apparent justification for pelvic surgery.—A. Cnl.

MALE GENITAL TRACT

Effect of Castration on Serum Phosphatases in a Case of Metastasizing Carcinoma of the Prostate. ABBOTT, L. D., JR., JAMES, G. W., and JARRETT, J. T. [Med. Coll. of Virginia, Richmond, Va.] *Virginia M. Monthly*, **70**: 195-200. 1943.

A case report. A study was made of serum acid and alkaline phosphatase over a 6 month period following castration in a case of metastatic prostatic carcinoma. Clinical improvement followed castration. Roentgenograms of metastatic bone lesions before and after castration are presented.—M. E. H.

Post-Mortem Findings in Carcinoma of the Prostate Following Castration and Diethylstilbestrol Therapy. A Case Report with Autopsy and Post-Mortem Tissue Acid Phosphatase Studies. GILBERT, G. G., and MARGOLIS, G. [Duke Univ. Sch. of Med., Durham, N. C.] *J. Urol.*, **50**:82-94. 1943.

This is a detailed case report of temporary improvement and subsequent failure in the control of prostatic carcinoma. Illustrations of microscopic and gross pathologic specimens, are included. Transurethral resection permanently relieved the urinary obstruction. Castration, diethylstilbestrol, and x-radiation were used in further treatment. In a review of the microscopic sections, from this case and from others, no changes in the neoplasm that could clearly be attributed to endocrine therapy were found. Changes apparently did occur, but correlation with clinical status and other features of the disease was at least indefinite. The phosphatase studies, however, did roughly parallel the course of the disease. The problem of failures following castration is discussed. The two usually proposed explanations for failures; namely, the existence of an extragonadal source of hormones, and the pronounced undifferentiation of the tumor are mentioned. The authors are of the belief that the final outcome from therapy cannot be foretold from the histologic appearance of the lesion.—V. F. M.

A Summary of Endocrine Effects in Advanced Prostatic Cancer. HUGGINS, C. [Univ. of Chicago, Chicago, Ill.] *New York State J. Med.*, **43**:519-521. 1943.

Inhibition of androgens by estrogens in prostatic cancer as opposed to castration is discussed. Endocrine castration is at first glance attractive, since it can be carried out without surgery and is financially economical. However, the author believes it to be unsound since the inhibition of androgens brought about in this way is partial and temporary. Furthermore, estrogen must be administered for long periods of time, and in many species this procedure in males is in itself carcinogenic. Although it has been shown that beneficial results occur in prostatic cancer

from both surgical castration and estrogen administration, bilateral orchidectomy appears to be the method of choice as a basic treatment in advanced or metastatic prostatic cancer.—J. L. M.

Prolan A in Diagnosis of Teratoma Testis. McDougall, T. G., and Graham, A. P. [Portland, Ore., and Maywood, Ill.] *West. J. Surg.*, **51**: 432-435. 1943.

A standard method of classifying testicular tumors is needed; up to the present no satisfactory classification has been offered. Prolan A, according to the present report, is a valuable aid in the diagnosis of teratoma testis. However, prolan A levels do not seem to coincide with particular histologic types nor do they indicate the relative malignancy of the tumor. An appreciable elevation in prolan A output may be expected in at least 75% of cases; 25% of cases in the series presented showed no elevation with proved malignancy. Conditions other than teratoma of the testicle may cause an increase in prolan A, though the level usually remains low—M. E. H.

A Report of a Series of Cases of Carcinoma of the Prostate Gland with Special Reference to the Importance of "Acid" Phosphatase Determination and Castration as a Means to Control the Malignancy. Worgan, D. K., and Toulson, W. H. [Sch. of Med., Univ. of Maryland, Baltimore, Md.] *Bull. School Med. Univ. of Maryland*, **28**:21-29. 1943.

In this series of 12 cases, acid phosphatase determinations on suspected cases of prostatic carcinoma were obtained and then repeated after bilateral orchidectomy so that the value of the test might be judged. Urinary symptoms were present in all cases, bleeding being the most important in 5. Eight patients showed signs of urinary obstruction upon admission. In this series, values of acid phosphatase greater than 3 units were considered suggestive of malignancy. Readings ranged from 4.2 to 35 units, with an average of 12.2. The number of patients is considered too small and treatment too recent to permit definite conclusions to be drawn, however, after bilateral orchidectomy all patients were definitely improved, especially the 5 suffering excruciating pain. In none had a cure been effected.—J. L. M.

The Medical and Surgical Aspects of Hypertrophy and Cancer of the Prostate. Young, H. H. [Baltimore, Md.] *Connecticut M. J.*, **7**:739-743. 1943.

The high incidence of carcinoma of the prostate has prompted the author to discuss the differential diagnosis and the operative procedures of choice. Reports made many years ago showed that carcinoma was present in 21% of cases with prostatic obstruction, and that carcinoma of the prostate was accompanied by benign hypertrophy of the lateral lobes in over 50% of the cases. The importance of the routine rectal examination is emphasized, and a plea made to abandon the exclusive use of transurethral resection for all types of prostatic obstruction, particularly the very large and the malignant.—M. E. H.

Urinary System—Male and Female

Rhabdomyosarcoma in the Lower Urinary Tract. Hunt, R. W. [New York Hosp., Flower and Fifth Avenue Hosps., New York, N. Y.] *New York State J. Med.*, **43**:513-517. 1943.

Rhabdomyosarcoma of the lower urinary tract is rare. Twenty-six cases were found in the literature; the tumor was in the bladder in 8 cases, and in the prostate gland in 18. More than 75% of the growths occurred in persons less than 40 years of age. The 2 additional cases reported here occurred in children 3 days and 2½ years old respectively. It is concluded that early radical operation will achieve the first successful treatment of this lesion.—J. L. M.

Wilms' Tumor: With Report of an Eight Year Cure. Ockerblad, N. F., and Carlson, H. E. [Kansas City, Mo.] *J. Urol.*, **50**:265-267. 1943.

An 11 week old male with a Wilms' tumor was treated by nephrectomy and postoperative irradiation. He is alive and well 8½ years later; this is the 13th known 5 year cure.—V. F. M.

Intrathoracic Tumors—Lungs—Pleura

Neuroblastoma of the Mediastinum. Bridge, F. [Mt. Sinai Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.*, **10**:426-428. 1943.

This case illustrates the small but important group of thoracic tumors arising from the sympathetic nervous system. An orange-sized tumor in a 2½ year old child was excised, and radiotherapy given postoperatively. There were no evidences of recurrence or metastasis 6 months later.—A. Cnl.

Metastatic Carcinoma of the Lung. Florman, A. L. [Mt. Sinai Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.*, **10**:429-430. 1943.

An orchidectomy for adenocarcinoma of the right testicle was performed upon a 2 year old child. Death from pulmonary metastasis occurred 2 years later.—A. Cnl.

A Case of Cervico-Thoracic Neurofibromatosis, Ginandes, G. J. [Mt. Sinai Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.*, **10**:414-419. 1943.

A child 4 years old presented extensive involvement of the cervicothoracic region with neurofibromatosis, the diagnosis being proved later at autopsy. Despite previous radiotherapy, symptoms of respiratory distress had appeared and tracheotomy failed to save the patient.—A. Cnl.

Adenoma of the Bronchus. Hennell, H. [Mt. Sinai Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.*, **10**:411-413. 1943.

This patient is one of the youngest on record with bronchial adenoma, having come under medical observation at the age of 9 years. The clinical features and course of the disease were observed for a period of more than 8 years. Repeated attempts to remove the tumor by bronchoscopy were unsuccessful. Lobectomy or pneumonectomy seemed to be indicated as the next step.—A. Cnl.

Excision of Teratoma of the Anterior Mediastinum. Neuhof, H. [Mt. Sinai Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.*, **10**:402-403. 1943.

A benign teratoma was removed from the anterior mediastinum of a child a little more than 2 years of age. Two and a half years after operation, x-ray examination of the chest is negative, and general health is entirely satisfactory, the child having gained 30 pounds. Compared with the problem of malignant teratoma, which is by no means infrequently encountered in adults, the problem of removal of a teratoma in a child is, according to the author, much simpler.—A. Cnl.

Huge Ganglioneuroma of the Mediastinum. Rabin, C. B. [Mt. Sinai Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.*, **10**:420-422. 1943.

Because of its size, a benign ganglioneuroma producing pressure effects was removed from a 5 year old child in 2 stages, 10 months apart. Three years after operation no recurrence of the tumor was found, and the patient was symptom-free.—A. Cnl.

Unusual Case of Carcinoma of Both Lungs Associated with Lipoid Pneumonia. Wood, E. W. [Presbyterian Hosp. and Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.] *Radiology*, **40**:193-195. 1943.

A case of bilateral alveolar carcinoma of the lung associated with lipoid pneumonia due to the inhalation of mineral oil is reported. The findings are compared to certain naturally occurring lung tumors in animals, and to pulmonary tumors induced in mice with fractions of natural mineral oil or by the intratracheal injection of hydrocarbons.—R. E. S.

Gastrointestinal Tract

Rectal Malignant Tumor in Childhood. Report of Two Cases. Bacon, H. E., Wolfe, F. D., and Archambault, R. A. [St. Luke's Children's Med. Center and Philadelphia General Hosp., Philadelphia, Pa.] *Am. J. Dis. Child.*, **64**:70-79. 1942.

The first patient, aged 3 years and 8 months, who had a reticulum cell sarcoma, survived 19 months after operation and irradiation. The second patient, aged 4 years and 7 months, who had an early adenocarcinoma, is still living at the expiration of 5 years and is clinically free of neoplastic disease. A long bibliography accompanies the report.—C. J. M.

Neurofibromatoses of the Colon, Small Intestine and Mesentery in a Child. Chalkley, T., and Bruce, J. W. [Univ. of Louisville Sch. of Med., Louisville, Ky.] *Am. J. Dis. Child.*, **64**:888-894. 1942.

Report of a case in a Negro girl, aged 8.—C. J. M.

Polyps of the Rectum and Colon in Infants and in Children. Kennedy, R. L. J. [Mayo Clinic, Rochester, Minn.] *Am. J. Dis. Child.*, **62**:481-488. 1941.

A report of cases of polyps of the rectum, the colon, or both, in 49 infants and children. The polyps were made visible by proctoscopic and roentgenologic methods. Treatment was by surgical methods, or by removal or destruction of the polyps with the aid of a proctoscope. Some of the lesions were frankly carcinomatous. All adenomatous polyps, except those found in certain cases of chronic ulcerative colitis, have carcinomatous potentialities and should therefore be removed or destroyed.—C. J. M.

Colloid Carcinoma of the Gastrointestinal Tract. Occurrence in a Boy Twelve Years Old with the Production of Pseudomyxoma of the Peritoneum. King, J. M., and Satory, J. J. [Milwaukee, and Wauwatosa, Wis.] *Wisconsin M. J.*, **42**:925-927. 1943.

A case report.—M. E. H.

Leiomyosarcoma of the Stomach. Report of Case. Lyons, C. G., and Schneider, M. [Veterans Administration, Hines, Ill.] *Am. J. Roentgenol.*, **49**:393-397. 1943.

A case report.—E. H. Q.

Primary Carcinoma of the Duodenum. Mantell, F. J., and Bradford, W. H. [Veterans Administration, Excelsior Springs, Mo., Veterans Administration, Brecksville, Ohio.] *M. Bull. Vet. Admin.*, **20**:108-109. 1943.

A report of a case in which carcinoma arose from an ulceration of the first part of the duodenum.—M. E. H.

Case of Partial Œsophagectomy for Carcinoma with Extrathoracic Gastro-œsophageal Anastomosis. Taylor, H. *Proc. Roy. Soc. Med.*, **37**:38. 1943.

Description of a case.—E. L. K.

Carcinoma of Œsophagus: Resection and Œsophago-gastrostomy. Thompson, V. C. *Proc. Roy. Soc. Med.*, **37**:37-38. 1943.

Description of a case.—E. L. K.

Transthoracic Resection of Carcinoma of the Cardia with Involvement of the Lower End of the Œsophagus, and Œsophago-gastrostomy. Tubbs, O. S. *Proc. Roy. Soc. Med.*, **37**:39-40. 1943.

Description of a case.—E. L. K.

Fibroma of the Stomach. A Case Report. Wiley, H. M. [Ellis Fischel State Cancer Hosp., Columbia, Mo.] *J. Missouri M. A.*, **40**:171-174. 1943.

Report of a case with a discussion of the pathologic features of the tumor, the surgical technic employed, and the differential diagnosis between these benign tumors and carcinoma of the stomach.—A. C.

Leukemia, Lymphosarcoma, Hodgkin's Disease

Monocytic Leukemia. (General Review of Subject.) Evans, T. S. [New Haven, Conn.] *Medicine*, **21**:421-456. 1942.

In the acute form of monocytic leukemia no treatment has proved to be of value. Transfusion has been widely used and has served to keep the patient alive for a short time. Occasionally a remission has been described following transfusion, but it seems probable that in such instances the transfusion is not the causative factor. X-ray has proved of little value. X-ray of the spleen and even splenectomy have been performed, but in the cases where success has been claimed for either measure, there seems to have been considerable doubt concerning the diagnosis. Theoretically, splenectomy is contraindicated in leukemia since it is in the spleen that excess of white blood cells is controlled by lysis and sequestration. In the chronic form of monocytic leukemia x-ray has been reported to have a beneficial effect.—J. L. M.

Classification and Diagnosis of Lymphoid and Allied Tumors. Foot, N. C. [Cornell Univ. Med. Coll. and New York Hosp., New York, N. Y.] *New York State J. Med.*, **42**:2220-2224. 1942.

A presentation and discussion of the classification used by the committee on diagnosis of the Lymphatic Tumor Registry.—J. L. M.

Monocytic (Histiocytic) Leukemia in Relation to a Previously Existing Sarcoma of the Skin. Case Report. Gueft, B., and Rosahn, P. D. [New Britain Gen. Hosp., New Britain, Conn., and Yale Univ. Sch. of Med., New Haven, Conn.] *Am. J. Clin. Path.*, **13**:516-526. 1943.

Generalized sarcomatosis of the skin is reported in an infant of 3 months, with later development of histiocytic leukemia. The cutaneous nodules were composed of more mature cells than those found later in the systemic

leukemia. Seven figures illustrate the gross and cytological details, and similar cases in the literature are cited.—J. G. K.

Adrenal

Sympathicoblastoma of the Adrenal Medulla with Metastases to Lungs, Lymph Nodes and Kidneys. Funke, J. [Atlanta, Ga.] *Urol. & Cutan. Rev.*, **47**:150-152. 1943.

This is a brief discussion in which the author mentions cases not strictly of either the Pepper or Hutchinson type. —V. F. M.

Paroxysmal Hypertension Caused by Pheochromocytoma of the Adrenal Gland. Adrenalectomy. Mencher, W. H. [Mt. Sinai Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.*, **10**:743-746. 1944.

This case represents a type of hypertension that is remediable by surgery. The paroxysmal hypertension and symptom complex depend on the discharge into the general circulation, of large amounts of pressor substance from a hormone-producing tumor of chromaffin type arising from the adrenal medulla. In this patient, after perirenal insufflation, x-ray examination revealed a right adrenal mass, and adrenalectomy was performed. A complete description is given of the clinical course of the disease and of the studies that were carried out.—A. Cnl.

Thymus

Effect of Removal of Malignant Thymic Tumor in a Case of Myasthenia Gravis. Poer, D. H. [Emory Univ., Atlanta, Georgia] *Ann. Surg.*, **115**:586-595. 1942.

The cases of myasthenia gravis reported in the literature are summarized. In 129 autopsies and operations performed on such patients, 71 thymic lesions in the form of persistence, enlargement, or tumors were observed. The author adds to this series a patient with a low grade carcinoma of the thymus, who was cured of myasthenia gravis after removal of the tumor.—E. A. L.

Thyroid

Carcinoma of the Thyroid. Gay, J. G. [Atlanta, Ga.] *South. Surgeon*, **11**:685-690. 1942.

The incidence of carcinoma of the thyroid in younger individuals is remarked. Attention is called to the fact that many malignant tumors are found in small glands. Since preoperative recognition of carcinoma of the thyroid is difficult and carcinoma usually arises in an adenoma, operative removal of the latter is urged. The types of thyroid carcinomas are discussed according to the degree of malignancy. A case of epidermoid carcinoma is reported.—E. E. S.

Carcinoma of the Parathyroid Gland. Meyer, K. A., and Ragins, A. B. [Cook Co. Hosp., and Cook Co. Grad. Sch. of Med., Chicago, Ill.] *Surgery*, **14**:282-295. 1943.

Report of a case with postmortem findings.—W. A. B.

Hürthle Cell Tumor of the Thyroid Gland. With Case Report. Reimann, D. L. [Sch. of Med., Univ. of Maryland, Baltimore, Md.] *Bull. School Med. Univ. Maryland*, **28**:93-98. 1943.

The Hürthle cell tumor is uncommon. It occurs most frequently in women; 15 of the 17 cases collected from the literature were observed in the female sex. The majority of the reported cases have been in persons in the fifth or sixth decade of life, however, one case was recorded in an infant 6 weeks of age. The signs and symptoms of hyperthyroidism are inconstantly associated with this type of tumor. It grows slowly but is of uncertain benignity. Those tumors that develop malignant characteristics are slow to metastasize. A typical example of the Hürthle cell tumor is reported.—J. L. M.

Multiple Tumors

Simultaneous Multiple Primary Malignant Tumors. Cashman, B. Z., and Cohen, M. [Elizabeth Steel Magee Hosp. and Univ. of Pittsburgh, Pittsburgh, Pa.] *Pennsylvania M. J.*, **45**:1183-1187. 1941-42.

Four cases of simultaneous multiple malignant tumors are presented. They include 3 examples of double malignancy and 1 of triple malignancy. The cases are as follows: 1) Squamous cell of the cervix and adenocarcinoma of the rectum. 2) Carcinoma of the breast and rodent ulcer of the neck. This patient also had a benign polyp of the cervix, and a year later an endometrial polyp was removed. 3) Adenocarcinoma of the uterus and sarcoma of the uterus. 4) Malignant papilloma in the bladder and both sarcoma and carcinoma in the uterus in addition to fibroid tumors of the uterus.—J. L. M.

Miscellaneous

The Diagnostic Problems of Early Cancer. Nineteenth Ludvig Hektoen Lecture of the Frank Billings Foundation, March 26, 1943. Craver, L. F. [Memorial Hosp., New York, N. Y.] *Proc. Inst. Med. Chicago*, **14**:410-420. 1943.

The author summarizes the symptoms of cancer of many organs and visualizes the defense in the war against cancer as having 3 objectives: (1) to rouse the patient to seek advice early; (2) to expedite the practitioner's recognition of the condition; (3) to make special facilities for diagnosis and treatment more generally available.—M. E. H.

Cancer from Pathologist's Angle. Dock, W. [Cornell Univ. Med. Coll., New York, N. Y.] *Northwest Med.* **40**: 369-372. 1941.

A discussion of the most advantageous relationship between the surgeon performing the biopsy and the pathologist handling the material. Some limitations of histologic diagnosis are presented. The author describes briefly his own views on the nature and causes of cancer.—E. E. S.

Cystic Lymphangioma of the Omentum Causing an Acute Surgical Abdomen. Hurwitt, E. S. [Mt. Sinai Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.*, **10**:294-297. 1943.

A review of the literature discloses that the great majority of the cases of omental cyst were discovered either incidentally, or at operation because of chronic symptoms such as abdominal distention. In the case reported the patient presented symptoms and signs of an acute surgical

abdomen. Nine cases were found resembling the present instance both clinically and pathologically.—A. Cnl.

Lipomata of the Omentum. Ikins, R. G., and Arbogast, J. L. [Lafayette, Ind.] *Indiana State Med. Ass. J.* **35**: 354. 1942.

A case report. The tumor, described in a 7 year old boy, was a benign growth weighing 2,300 gm., easily removed from the omentum. Symptoms were mild until thrombosis of vessels leading to the mass occurred, causing hemorrhage and severe abdominal pain. Recovery was uneventful following resection of the tumor.—E. E. S.

Malignant Tumors Developing in Sacrococcygeal Teratomata. Lisco, H. [Johns Hopkins Med. Sch. and Hosp., Baltimore, Md.] *Ann. Surg.*, **115**:378-389. 1942.

Malignant tumors developing in sacrococcygeal teratomas have occasionally been observed. Two case studies are submitted. Ten similar cases found in the literature are reviewed. Early surgical removal of sacrococcygeal teratomas is advocated, with careful microscopic examination for malignant changes of the tissue at the point of attachment of the tumors.—M. R. D.

Tuberculous Lymph Nodes of the Neck Involved in Metastatic Carcinoma. Meyst, C. H. [Veterans Admin., Wood, Wis.] *M. Bull. Vet. Admin.*, **20**:220-221. 1943.

A case report.—M. E. H.

Liposarcoma. Report of Nine Cases. Moreland, R. B., and McNamara, W. L. [Veterans Administration Facility, Hines, Ill.] *Arch. Surg.*, **45**:164-176. 1942.

Case reports.—G. H. H.

Tumors of the Neck. Peterson, E. W. [New York Post-Graduate Med. Sch. and Hosp., New York, N. Y.] *Am. J. Surg.*, **61**:350-359. 1943.

A general discussion and description of operative technics with special reference to congenital cysts and fistulas.—W. A. B.

Should the Cancer Victim Be Told the Truth? Seelig, M. G. [Barnard Free Skin and Cancer Hosp., St. Louis, Mo.] *J. Missouri M. A.*, **40**:33-35. 1943.

The reasons why the patient should not be told that his disease is cancer are discussed.—A. C.

The British Empire Cancer Campaign has sent the following statement to the *Lancet*, *The British Medical Journal*, *The Medical Press and Circular*, and *Nature*:

"Ever since the British Empire Cancer Campaign was founded, it has been one of its most important duties to pass in review new suggestions which are made from time to time as to the cause and treatment of cancer. In the past the conclusions arrived at have not always reached the medical profession. At the present time, when practitioners are seeking enlightenment about cancer it is more than ever important that the medical profession should be able to obtain authoritative information concerning the value of various suggested remedies, and of any theories as to causation. The Campaign, therefore, will be willing to communicate its opinion on any new form of treatment on which it has information.

"The Campaign will continue to investigate methods of treatment and theories of causation and is willing to undertake or to promote research into such subjects, provided the following conditions are fulfilled:—

(1) That, in the opinion of the appropriate expert committee of the Campaign, such a subject offers any prospect of advancing the solution of the cancer problem.

(2) That the fact that a theory or suggested treatment is being investigated by the British Empire Cancer Campaign shall only be disclosed with the consent of the Campaign.

(3) That the Campaign reserves to itself the right to publish, in an appropriate manner, the conclusions reached, whether favourable or otherwise.

(4) That, in the case of theories concerning causation, all available information shall be furnished by the advocate of the theory on the scientific basis and the experimental data which shall be so detailed that exact repetition of the experiments can be carried out by experts in the field of research concerned.

(5) That, in the case of methods of treatment, the precise nature, composition and method of administration of the suggested remedy shall be disclosed and that the evidence shall be collected in accordance with the safeguards as to scientific accuracy which experience has shown to be essential, namely:—

  (a) That cases shall be of proved cancer, in so far as proof is practicable, preferably by histological examination. For choice, they should be cases affecting so-called 'accessible' organs, e.g. skin, breast, cervix uteri and mouth.

  (b) That every case treated shall be recorded, whether the result is favourable or otherwise.

  (c) That the clinical records, including 'follow-up' shall be as full as possible.

(6) That, in the case of a treatment based on experiments, the Campaign reserves to itself the right to confirm the results of such experiments before attempting clinical trials of the remedy.

"The Campaign will be happy to arrange for medical men to discuss their hypotheses and experiences with appropriate experts in the field concerned."—E. L. K.

Book Review

BIOCHEMIE DER TUMOREN. Hans v. Euler and B. Skarzynski. Ferdinand Enke Verlag, Stuttgart, 1942. 260 pages.

The current emphasis on the biochemical aspects of the cancer problem makes the appearance of a monograph dealing with this subject an event of interest. The senior author of this book is the distinguished Swedish biochemist whose contributions to the knowledge of the coenzyme factors (adenine nucleotides) in fermentation have won him world renown. In collaboration with Skarzynski, his work in the cancer field has been largely concerned with the dehydrogenase systems in the Jensen rat sarcoma. The book that these two investigators have written on the biochemistry of cancer is apparently intended to be inclusive to 1940, although a few references in 1941 have been included both in the text and in a brief appendix of more recent work. The unevenness in the reporting of the work in 1941 is most likely to be attributed to difficulties in the communication of the literature of science in a world at war.

The book is divided into the following chapters: Origin and Classification of Tumors, General Conditions for Tumor Growth, Experimental Cancer Studies, Chemical Components of Tumors, Enzyme Systems and Metabolism of Tumors, Metabolism of the Cancerous Host, Carcinogenic Substances, Influence of Hormones on the Formation and Development of Tumors, Tumor Viruses, Mutation and Origin of Cancer Cells by Radiation and by Chemical Agents, Inhibition of Tumor Development by Chemicals and by Tissue Extracts, Nutrition and Cancer, Natural and Induced Immunity, and Chemical Diagnosis of Cancer. The reader will find many of the chapters well written, lucid, and informative. This is particularly true of the introductory chapters and of those dealing with carcinogenesis, viruses, inhibition of tumors, and immunity. In a few places the authors might have been more explicit; *e.g.,* in referring to induced tumors of the brain, spleen, or liver, the tissue from which the particular tumor arose should be designated. The less satisfactory parts of the book are the chapters concerned with the chemical components and enzymes in tumors, with radiation, and with nutrition. These sections are rather unevenly presented. Fourteen pages in the chapter on enzymes are devoted to fundamental oxidation mechanisms and only one line, and that of curt dismissal, to the work of Warburg. The deliberate neglect of the huge body of outstanding work on the respiration and glycolysis of tumors by Warburg and by the English and American schools detracts from the value of the book. Perhaps future editions may include this material. No mention at all is made in the chapter on enzymes to the work of the Kinosita group, who, using hepatoma and normal liver, made the earliest comparisons of the enzymatic activity of a tumor and its tissue of origin. When one considers the personal interests of the authors of this book, it is surprising to find the subject of enzymatic catalysis treated altogether so cavalierly. The chapter on radiation includes an extended discussion on theories of mutation with little or no mention of high voltage therapy. The topic of nutrition in cancer is treated in desultory fashion and is largely based on the few observations on avitaminosis in the clinical literature. An omission that might be noted in the chapter on tumor inhibition is that dealing with the use of bacterial toxins.

No doubt it is impossible for an author of a book on cancer, particularly one dealing in broad biochemical terms, to satisfy every reader; the mere collection of all the vast amount of data in this field is a staggering task, which must elicit immediate sympathy and admiration. To place this material within the covers of a book, the author must choose one of three courses, namely, (a) to present all the material, contradictory and otherwise, without comment; (b) to present all the material, and, considering the controversial data critically, to express considered opinions; and (c) to present only those data that appear to be sound and carefully drawn. With the exception of the omissions noted, v. Euler and Skarzynski have followed course (b). That there is some value in this method cannot be denied. It is interesting to have conflicting points of view so lucidly expressed and sensibly resolved as these authors have done. Singularly enough, however, this sensible resolution most frequently takes the negative turn; mostly it is concerned with the dismissal of many of the dubious theories and practices with which the course of cancer research has been strewn. Too frequently, work on the cancer problem, particularly in central Europe, has taken the philosophical view, and has combined biochemistry and clinical cancer in much the same fashion as Mr. Pott's erudite and ingenious friend synthesized Chinese metaphysics. The theories of immunity and the foundations of diagnostic practices have been examined by v. Euler and Skarzynski and nearly all have been declared valueless. The question arises therefore, whether these descriptions have any other than historical value and whether a monograph on the biochemistry of cancer should be largely a history of buried hypotheses. Modern monographs on, say, biology, do not devote very much space to quarreling with the idea of spontaneous generation. Does it serve any useful purpose, therefore, to disinter the kind of thinking that devised the Freund-Kaminer, the Waldschmidt-Leitz, the v. Christiani hypotheses, or the kind of experimentation that saw significant differences amounting to 0.06 in the pH of normal and of cancerous blood (when the liquid junction potential causes uncertainties in excess of this)? A history of many errors, entertaining though it may be, is not always helpful to future progress. It is possible that a thinner volume, embodying only the plain, unadorned, dull facts that unambitious cancer research has brought forth, may be more constructive than the encyclopedic type of monograph.

A few minor points in the book under review may be briefly mentioned. Contrary to the authors' statement in their Foreword, theirs is not the first book on the subject in the German language, for that of Wilheim and Stern preceded it by several years. Very frequently names are mentioned throughout the text without adequate reference given, and are sometimes misspelled. References to names are inconsistent in giving the initials.

JESSE P. GREENSTEIN